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JUNE, 1952

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EDITOR'S VIEWPOINT

The tactile sense of the dental apparatus is generally recognized but where it is centered and its functions in mastication had not been intensively investigated. Manly, Pfaffman, Lathrop, and Keyser (p. 305) studied the ability of individuals with natural and artificial dentitions to distinguish thickness of disks, hardness of objects, pressure of fibers, and changes in food texture. Obviously, those sensations dependent on the periodontal membrane reaction in the natural dentition will be greatly reduced in wearers of artificial dentures. Tactile sense and ability to distinguish food texture are greatly reduced with loss of teeth and hardness of relatively hard objects is not as accurately estimated by denture wearers. The tactile sense test, using fibers of varying stiffness, might well be adapted for periodontal investigations.

The amount of tissue per mouth involved by gingival inflammation did not appear to be greater in young men from the Southwest, although the DMF was lower among young sailors from the Southwest. Moreover, Massler and Ludwick (p. 313) found no difference in caries experience and in extent of gingival envolvement in young men from communities of different size. Of course, periodontal disease is recognized as increasing with age and it is probable the irritating factors produced by caries in the mouths of young people might reflect to a greater degree several years later than they would among adolescents.

Ludwick and Massler (p. 319) found no relationship between tobacco smoking and gingivitis as indicated by the PMA index but did find some correlation in the very few (20) cases of ulcero-membraneous gingivitis encountered. To draw conclusions on the basis of 20 cases would be unjustified. A very interesting observation was the direct relationship between caries history (DMF) and tobacco consumption. Many had assumed an inverse relationship. Again, to accept this as cause and effect is unsound. These young men have been developing cavities over a period of many years, while their smoking is a relatively recent habit. Such findings suggest to us the danger in using surveys of this type to draw conclusions without a careful and thorough consideration of the modus operandi and the scientific possibilities. The authors are justifiably cautious.

The PMA has been proposed and used (see Massler and Ludwick, p. 313) for mass studies of periodontal disease but its validity has been questioned because it does not differentiate types of pathologic change. However, even as a screening index, it should have some value in mass studies. Each of 8 dentists, as reported by Sandler (p. 323), after several weeks' experience with the index, examined 10 patients. These results were markedly different and probability studies suggest that while the method may be useful for group comparisons by one examiner, its application to groups by different examiners should be made "with extreme caution."

Turkheim (p. 326) found that tobacco smoke inhibited the growth of some microorganisms in vitro. The author directs attention to the fact that the effect in vivo has been considered rather inconsequential. The difference in effect of any agent in the test tube or on the Petri dish may be far different from that in the mouth. In vitro studies of agents proposed to reduce bacterial activity are often cited as acceptable basis for their use in the oral cavity, although bacteriologists and pharmacologists have long recognized the inadequacy of the phenol test. Among the factors that might make the difference in the mouth and in the test tube are the short contact between tobacco products and bacteria due to the important "cleaning mechanism" in the mouth, the possible neutralizing effect of salivary components and the more favorable important "clearing mechanism" in the mouth as well as the well-recognized differences in action in cellular environment.

Ring (p. 329), after experimental wounding of rat gingivae, demonstrated higher alkaline phosphatase concentration in the healing regions. In the epithelium, this

phosphatase appears to be associated with the keratinization which accompanies the healing process. Activity of endothelium during clot organization, fibroblasts during the formation of fibers, and macrophages in phagocytosis are also associated with increased concentrations of alkaline phosphatase as identified by the Gomori method.

From examination of 1570 Naval men, Cross (p. 335) predicts the dental needs per 1000 men. Because of conjecture concerning the number of dentists needed per population group, both in civilian and military life, such data are valuable. It is unfortunate that only one-third of the men were examined by use of bite-wing radiographs and that the data for these men are not treated separately from those of the other two-thirds. The statement that radiographically observed enamel lesions not large enough to restore were not counted is open to discussion. If an area of decalcification is observed by the x-ray method is it not a carious lesion and should it not be predicted as one requiring dental care? However, since the author would not fill such areas his data are not invalidated by this decision.

Many individuals and groups are evaluating dental caries control techniques by studies of groups of children. Too often more attention is paid to the numbers of individuals in the groups than to the accuracy and care in examination of each individual. Blayney and Greco (p. 341) show an amazing increase in the numbers of carious lesions revealed when radiographic examinations are added to clinical examinations. Relatively small differences in the average number of lesions per child may greatly influence the results when statistical methods are employed. It is often assumed that where large groups are employed the errors cancel out, but in research such a possibility is not so certain as to be acceptable. No statistical evaluation can substitute for careful and thorough examination or, expressed differently, mathematical accuracy cannot correct the errors introduced by inaccurate clinical studies. The great numbers of cavities revealed in different percentages at different ages by radiographic evaluation should give warning to those who consider such thorough studies unnecessary in research.

Hill, Blayney, and Wolf (p. 396) have now tabulated the caries experience rates for 6 to 8-year-old Evanston children exposed to artificially fluoridated water for periods from 12 to 58 months. Comparisons with the base-line group examined in 1946 groups show an increase in caries in the deciduous teeth of the fluoridated group (4.49 per cent) which is not considered significant, and a reduction in the DMF permanent teeth (46.42 per cent) which is considered significant. A careful study of these data suggest that a longer period of study is essential if the effects of artificially fluoridated waters are to be mathematically evaluated.

Boyd, Cannon, and Leighton (p. 354) direct attention to a very important cause of error in studies of dental caries control methods. If, at the same time that a general caries control procedure were introduced an incentive for more dental care were given, it is possible that appreciable error would be introduced. Since it is most impractical to limit caries control studies to untreated groups, investigations should consider weighing the value of incidental dental restorations.

The relationship between dextrinization of starch by enzymes and temperature has been investigated by Turner and Crowell (p. 359). They found marked differences in dextrinization times at different temperatures and they direct attention to the need for control of temperature in studies of starch breakdown by components in the saliva.

Periodic oral examination of the teeth of hamsters and other rodents, as well as carrying out experimental procedures in their mouths, is difficult. Johansen (p. 361) is able to perform examinations and treatment procedures in the mouths of live, unanesthetized rodents with the use of a mechanism and technique described. The apparatus has possibilities of wide application.

The physical properties of materials used to restore parts of teeth have been intensively investigated. Since the work of G. V. Black few studies have been made on the physical properties of dentin and enamel, the substances being replaced. It is obvious that certain of these physical properties of tooth substance are as important

as those of the restorative materials if external displacement and internal deformations are to be avoided. Peyton, Mahler, and Herschenov (p. 366) report the preliminary results of investigations of the physical properties of dentin and point out some of the difficulties encountered in testing the biologic material.

The interesting problem of the existence of tricalcium phosphatase hydrate in calcified tissues and its possible relationship to hydroxyapatite was investigated in an intriguing manner by Posner and Stephenson (p. 371). They enlarged the crystals in a hydrothermal bomb and submitted them to chemical analysis, x-ray diffraction, optical studies and density measurements. They postulate the cause of the differences between hydroxyapatite and tricalcium phosphatase hydrate. This is the first report from the A.D.A. cooperative research on the physics and chemistry of hard dental tissues at the National Bureau of standards.

There is probably no more moot subject in the field of dental histology than the presence or absence of nerve fibers in the dentin. Utilizing two different techniques, Powers (p. 383) demonstrated processes in the dentin which she interprets as nerve fibers. The author, apparently, is well aware of numerous claims that there are no nerves in the dentin and presents photographic evidence of fibers taking silver nitrate stain progressing from the pulp into the dentin and appearing as nerves. While this will probably open the controversy for further discussion, it is also hoped that it will lead to further study and possibly resolution of this important question.

Yardeni (p. 393) found that dentin lost weight faster than did normal enamel in lactic and buffers at various pH. A lag period in decalcification (as measured by loss of weight) was demonstrated for enamel caps. The use of gravimetric methods for determination of decalcification rates of tooth substance is of questionable value. Yardeni points out one important source of error, i.e., inhibition and retention of fluid after decalcification has proceeded beyond certain stages. Other possible sources of error are discussed.

From Fleming's (p. 398) experimental study, methylcholanthrene appears to have greater effects on the young epithelium than on connective tissue, but it does not always prevent formation of some hard dental tissues. The ameloblasts formed keratin, sometimes without differentiation into squamous cells. Fleming, observing that the keratinized cells were very elastic and adherent, suggests the role of keratinized ameloblastic material in binding enamel rods. The changes observed have significance in understanding of the process of odontogenesis.

The relative influence of the facial musculature on the development of the dental arch form and facial skeleton has much interest for anatomists, orthodontists, and pedodontists. Horowitz and Shapiro (p. 412) approached the problem experimentally, measuring facial and arch dimensions after removal of various portions of the facial musculature. Only complete removal of the orbicularis oris, of the operations performed, resulted in significant changes in the skeleton and then they were limited to the premaxillary and nasal areas. The use of this muscle in sucking and mastication offer some basis for further consideration of its influence in maxillary discrepancies.

Myers, Jennings, and Becks (p. 416) describe a method for rapid radioautographic studies of hard and soft tissues. Such a technic is most valuable with radioisotopes of short half-life.

The problem of antibiotics as caries control agents still remains enigmatic. Stephan, Fitzgerald, McClure, Harris, and Jordan (p. 421) found penicillin by far the best of five antibiotics for controlling dental caries in rats but failed to correlate control of dental caries with reductions in bacterial population. They observed that the most effective inhibitors of gram positive organisms were the best caries inhibitors. It is interesting that fracture of cusps in animals on antibiotics in many instances was not followed by dental caries, a rare finding in control animals.

The separation of bacterial clumps has a great effect on the number of colonies per c.c. cultured from salivary samples. Williams and Eickenberg (p. 428) found about 10 minutes of sonic vibration (9000 cycles/sec.) markedly increased salivary bacteria counts. Since the noted increase was quite marked it should be pointed out that new values for caries immunity and resistance

will be needed if the method is generally employed. The salivary lactobacillus index is a useful tool, but like all biologic tests, must be re-evaluated from the clinical standpoint each time it is modified.

The effect of ascorbutic diet on the dentin of teeth is well recognized. There has, however, been some question raised concerning the effect of scurvy on enamel of guinea pigs. Kalnins (p. 440) reports a study in which he found that the primary changes in the enamel are due to retardation and complete cessation of the development of dentin in the molars and that secondary and non-typical scorbutic changes occur as a result of detachment or pressure atrophy of the ameloblast caused by the bending of the tooth, or the transmission of masticatory forces into the formative portion of the teeth. This study tends to confirm other work showing the inter-relationship of amelogenesis and dentinogenesis. In addition, Kalnins believes that his work demonstrates that the maturation phase of enamel formation is determined by the activity of the ameloblasts and not by function of the pulp at the dentinal enamel junction.

H. B. G. R.

ORAL SENSORY THRESHOLDS OF PERSONS WITH NATURAL AND ARTIFICIAL DENTITIONS

R. S. MANLY, CARL PFAFFMAN, DONALD D. LATHROP, AND JOAN KEYSER

From the Laboratory for Oral Physiology, Tufts College Dental School, Boston, Mass., and Department of Psychology, Brown University, Providence, R. I.

IT IS well recognized that the sensory mechanisms of the mouth fulfill protective and selective functions.[1] Oral sensations are obviously needed during mastication, as was observed in a study with a subject before and during mandibular block anesthesia. His chewing ability was diminished during anesthesia. Some fairly large particles of food were missed during the mastication. The subject stated that he was unable to sense the location of unchewed food, and hence could not bring it to the occlusal surfaces of his molars for mastication.

Persons who wear complete dentures lose tactile sensations which are derived from the periodontal membrane. It is possible that the sensory cues from food become impaired after the transition to dentures. Perhaps blunted sensations would cause manipulation of food to be less efficient, which would explain why the masticatory efficiency of denture wearers is only one-fourth that of the normal dentition on the average, and why it varies widely from one individual to another, without apparent cause.[2]

This investigation is concerned with certain sensory processes mediated by natural or artificial teeth. The tests selected represent a compromise between those which can be estimated readily and those most likely to be related to masticatory function. Thresholds for the discrimination of thickness, hardness, light touch, and texture were studied in persons with normal and artificial dentitions. The purpose was to compare how well persons with natural and artificial dentition could discriminate these stimuli, in order to learn whether such discrimination among denture wearers is impaired. If there was any impairment, it might be partly responsible for low masticatory efficiency of some denture wearers.

METHODS

Size Judgment Between Anterior Teeth.—Rods of boilable lucite one inch in diameter were obtained from the Dupont Company. Disks were cut from these and machined and polished to thicknesses of 5.00, 5.25, 5.50, and 5.75 mm. ± 0.01 mm. During a test each disk was placed horizontally between the subject's incisor teeth and held by the examiner while the subject bit gently but firmly upon the disk. Each subject was given a familiarization trial with each disk, presented in the order of thinnest to thickest. During an actual test, subjects were presented disks in the following order: ADCB CABD DBAC DBCA BCAD CDBA DCAB CB. He indicated his response by writing plus on paper whenever he believed the disk was one the thicker two (C or D) and by writing minus in case the disks were perceived to be one of the thinner two (A or B).

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The subject was then given the series in reverse, i.e., thickest to thinnest, and another thirty presentations were made in exactly the reverse order as that above. From this data the percentage of "thicker" responses for each disk was tabulated and plotted on probability paper against the logarithm of the thickness of the disk. The threshold for sensitivity was calculated by drawing a smooth curve through the points and reading off thickness of test objects at the 25 per cent and 75 per cent intersections. The difference in thickness, divided by two, represents the threshold. This threshold represents the ability to detect differences in the thickness of the test objects. It is known as the method of single stimuli.[3] The value obtained is a measure of the slope of the theoretical straight line joining points representing percentage of the responses. The slope would be zero at the 50 level if the subjects were simply guessing; the slope increases as the subjects become able to discriminate between test objects.

Hardness Judgments Between Anterior Teeth.—Estimation of hardness was made by a very similar procedure; in this instance the disks had the same thickness (5 mm.) but differed in hardness.* The initial tests were made with slabs of white rubber stock, varying in hardnesses from 1.02 mm. to 2.31 mm. (readings on a Pusey Jones plastometer with 1 kg. load on a ¼ inch ball for two minutes). This range of hardness was found to be too great and a second series was obtained from 1.09 to 1.17 mm. in hardness. This series presented the proper order of difficulty for the subjects in that no perfect scores were obtained, yet all subjects were able to discriminate between some of the disks. A series of disks was then prepared using glass, steel, aluminum, and lucite, whose respective hardnesses were 448, 233, 124, and 1.5 units as determined by a Knoop indentometer with a 600 gram load. The thickness and size of the disks were identical. This series proved to be unsuitable since nontactile cues were obtained; for example, the steel gave auditory, thermal, and gustatory cues. In order to avoid this difficulty a graded hardness series was prepared from a formulation of a high-resin copolymer mixed in varying ratios with GRS. Four samples, designated a, b, c, and d, initially had Knoop hardnesses of 0.67, 1.76, 2.93 and 4.16, respectively. Disks were prepared by softening the sample in an oven, compressing to final thickness, and punching out disks after the material had cooled. The hardness readings were then made and reported as averages taken on single disks. Later it was found that the numerical values for hardness varied, depending upon slight changes in the technique used to bring the resin to proper size. The order of hardness of the disks, however, did not change.

Tests were also performed on denture patients with topical anesthesia. Dentures were removed, cleansed, and dried. The total denture-bearing area was wiped dry and painted with a cotton applicator containing Mann's topical anesthetic solution, and the dentures were quickly reinserted. Tests were performed within fifteen minutes, the duration of anesthesia of the gingiva.

Tactile Sensitivity of Teeth.—A series of Von Frey tactile hairs were prepared from nylon fibers, 7 to 20 thousands of an inch in diameter. These were cemented at right angles to the ends of plastic rods and adjusted in length to

*Samples for hardness were obtained by courtesy of D. D. Wright of the Hood Rubber Company.

give a range of force from 0.7 mg. to 125 Gm. ± 10 per cent. The scale was logarithmic with each fiber exerting 50 per cent more force than the next softer one and 33 per cent less force than the next harder filament. Teeth were tested repeatedly in ascending and descending series, subjects responding with hand signals when they could detect the touch. In nearly every instance the end point was definite. The least detectable stimulus was recorded as minimal threshold. This type of threshold is known as absolute, and differs from the differential threshold used in the two previous procedures.

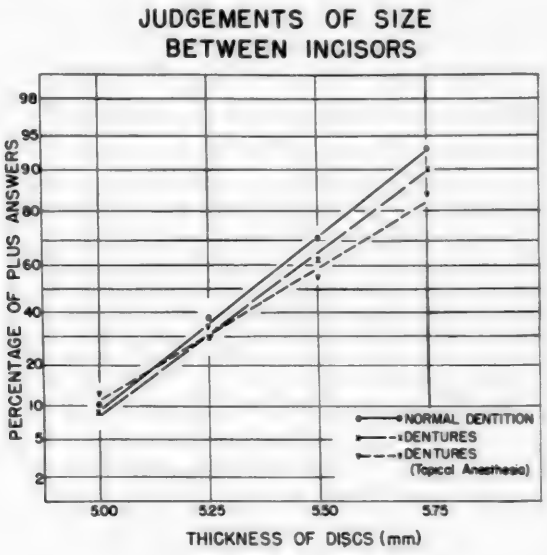

Fig. 1.

Estimation of Threshold for Detection of Changes in Texture.—In the previous tests it is probable that the thresholds given are for one or two primary modalities. The size and hardness judgments most likely combine position and deep pressure estimation while touch is probably a single modality. In mastication of food, however, many of the sensations experienced are concerned with the texture of the food, which probably combines estimations of a number of the properties including hardness, toughness, friability, vibration, and sound. No literature could be found as a guide to selection of techniques for approaching this problem. Trials were made with pulverized breakfast food, but in every instance the preparation and maintenance of reproducible standards could not be achieved.

Finally a parameter was selected which involved the detection of graded quantities of dentifrice grade calcium carbonate suspended homogeneously in a bland pudding. Burnett's brand of instant pudding, vanilla flavor, was prepared according to instructions by mixing the powder with milk. To different batches of this were added 0.1, 1.0, 2.0, 2.9, 4.8, 7.4, 9.1, 17, 23, and 29 per cent calcium carbonate.* This gave three series of puddings designed for

*Whittaker #64, Free Flowing Heavy $CaCO_3$ with mean particle size reported as 12.5 microns from Whittaker Clark and Daniels, Inc., 260 W. Broadway—New York City.

different thresholds; one included the 1.1, 1, 2, and 2.9 per cent, another the 2.9, 4.8, 7.4, and 9.1 per cent and the third, the 9, 17, 23, and 29 per cent. For practical reasons the tests were abbreviated somewhat from that used for the presentation of disks. The order of presentation in each case was ADCB DACB A. A total of ten individuals with natural dentitions and ten with artificial dentitions was studied.

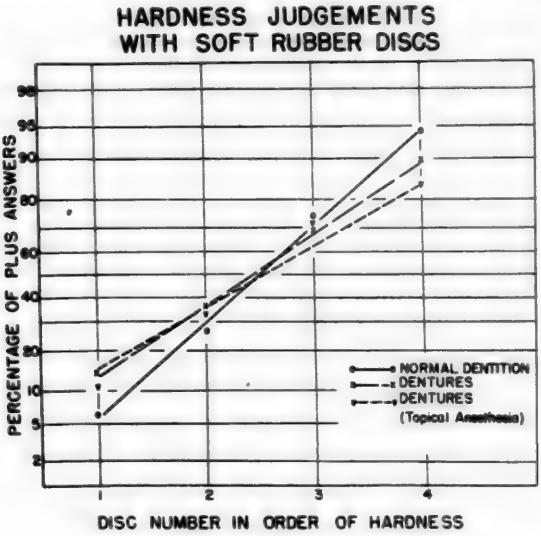

Fig. 2.

In this test the subject was instructed to report whether or not he could detect the "grit," and was asked to make his estimations by rubbing the pudding between his teeth. This type of judgment would be known as an absolute threshold, because the subject reports when he can first detect the presence of the substance and does not attempt to judge differences among the concentrations presented. The series for the denture cases was not broad enough, and six of the thresholds for individuals fell beyond its limits. Hence the judgments could not be combined and solved graphically. Instead the judgments of each individual were tabulated, the threshold estimated roughly, and grouped into four categories of $CaCO_3$ concentration.

RESULTS

In Fig. 1 is presented the thresholds for size judgment on plastic disks. The curve for normal dentitions is the average of test runs on six subjects. Six denture wearers were tested both with and without topical anesthesia by means of Mann's solution. It can be seen that there is no significant difference among the sensitivities or thresholds of the three groups. The threshold sensitivity amounts to 0.18 mm. or 3.3 per cent of the total thickness of the disk. Since the thresholds are not affected by the substitution of dentures for natural

teeth, even when the tactile sensation of the tissue supporting the denture has been reduced by topical anesthesia, it is probable that the judgment of size is accomplished from proprioceptive sensations originating in the temporomandibular joint or the muscles of mastication. There is no indication that the size judgment of persons is handicapped by transition to the wearing of dentures. Unpublished studies by Lunin and Levy led them to the opinion that proprioceptors in muscles, tendons, and/or joints are involved in thickness judgment between teeth.[4] Eighteen subjects were tested for their ability to detect 1 mm. differences in 5, 8, 12, and 17 mm. disks when the disks were placed between anterior teeth. Thickness was judged more accurately between teeth than between fingers, but the marked difference in experimental method between that and the present study makes Lunin and Levy's data of questionable relevance.

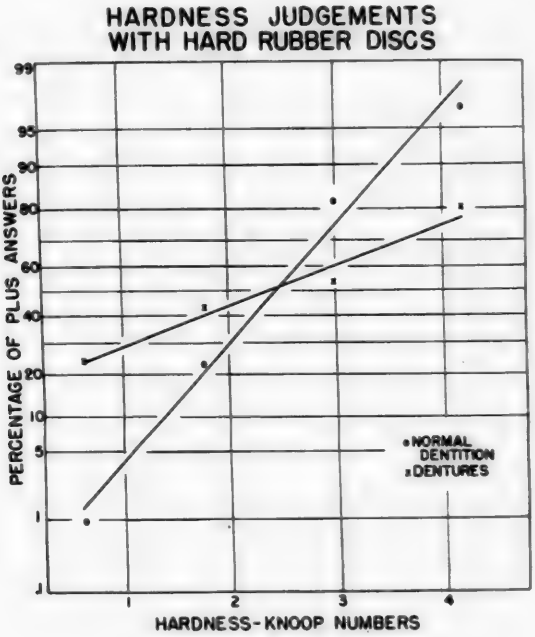

Fig. 3.

The results with hardness judgments on soft rubber disks gave a similar conclusion (Fig. 2). There is only slight difference apparent between the normal dentition group and the denture group, whether or not the tissues of the denture group had been subjected to topical anesthesia. Here again is evidence that the discrimination of hardness is achieved without need for a periodontal membrane and hence the denture wearers suffer no great handicap.

When tests were made with hard rubber disks, denture wearers and natural dentition cases showed different thresholds. The findings with sixteen normal dentition cases and thirteen denture wearers are presented in Fig. 3. The curves are strikingly different. The threshold for denture wearers is nearly

three times that of the natural dentition cases. This finding is in contrast to the results with the soft rubber disks and probably can be attributed to the different relative amounts of motion of the mandible in biting on soft and hard rubber test objects. It is generally recognized that the compressibility or displacement of tissues permits a slight movement of the denture relative to the mandible or maxilla. When the subject bites on soft rubber this movement is small compared to the distance through which the teeth move when sinking into the rubber disk. With hard rubber the teeth penetrate the disk less than 0.1 mm. and the instability of the denture becomes a factor which limits precision of judgment.

Tactile Sensitivity of Occlusal and Incisal Surfaces of Teeth.—The mean thresholds for ten subjects with natural dentitions are presented in Fig. 4. The sensitivity of anterior teeth is about ten times as great as that in posterior teeth; the mean threshold for the biting surface of anterior teeth is about 1 Gm. whereas that for first molars is 8-10 Gm. This represents the minimum force which can be detected when the force is applied against the biting surface of the teeth in line with the long axis of the teeth. Stewart reported findings on tactile sensitivity of two hundred and sixty teeth of subjects 18 to 29 years of age.[5] His data were given in pressures of 7 to 50 Gm. per square millimeter with a mode at 17 to 21 Gm. per square millimeter. These results cannot be compared with our thresholds because of the difference in techniques.

TABLE I
NUMBER OF SUBJECTS ABLE TO DISTINGUISH PRESENCE OF $CaCO_3$ AT DIFFERENT CONCENTRATIONS

Concentration	0.1-2.9	3-9	9-24	Over 24
Natural dentition	9	10	10	10
Denture patients	0-3	4	6	10

A similar study of several cases was made with the force applied to the labial surfaces of the teeth with calibrated fibers. The threshold forces in this direction averaged about 0.5 to 0.6 Gm. for incisors and 1.8 to 2.4 Gm. for first molars. The sensitivity to the lateral forces appeared to be two to five times as great as for axial forces. Eight denture wearers were then tested for thresholds to forces applied to occlusal surfaces of the first premolars of the mandibular dentures. The subjects were so insensitive that additional hairs with greater stiffnesses had to be prepared. The stiffest fiber available exerted 125 Gm. Five subjects were insensitive to this force; one reacted to 56 Gm. and the other two to 83 Gm. The average threshold for denture wearers is apparently over ten times as great as the threshold for persons with a natural dentition. It can be concluded that tactile sensation from food is greatly impaired by the transition to dentures.

Judgment of texture by ten natural and ten artificial dentition cases is presented in Table I. The number of subjects able to distinguish presence of $CaCO_3$ at different concentrations is compared for natural dentition and denture cases. Nearly all natural dentition cases could detect 2.9 per cent $CaCO_3$ or less, whereas the majority of denture cases required over 9 per cent $CaCO_3$ for detection. The denture cases had a very wide range in threshold which

extended beyond the concentrations of $CaCO_3$ which were originally selected; three would have needed tests with more than 29 per cent $CaCO_3$ and three would have required tests with less than 9 per cent. Hence the thresholds cannot be specified, yet it is clear that the sensitivity of texture judgment for the majority of denture cases is considerably less than that for normal dentition cases.

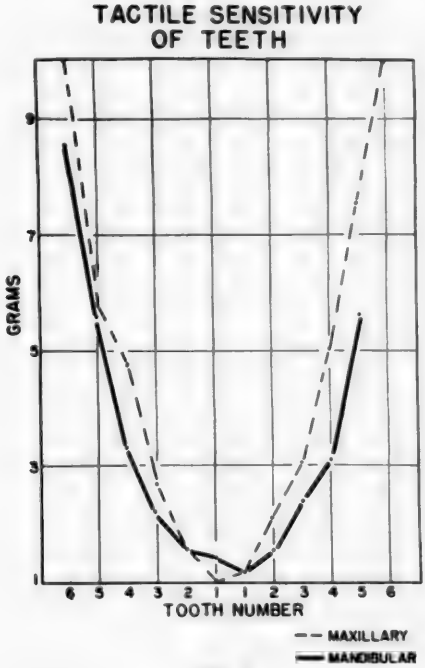

Fig. 4.

DISCUSSION

When the results of the five tests are considered together, they make a consistent picture of the differences between sensory thresholds for teeth of the natural and artificial dentition cases. The transition to dentures does not impair the sensations of size and hardness of the food bolus, because these sensations are perceived by proprioceptive impulses which originate in muscles, tendons, or joints rather than the periodontal membrane. The relative motion between the denture and the mandible during chewing has been shown by Boucher[6] to be about 0.1 mm. With a gentle closure the relative motion is presumably even less, and it is insufficient to interfere with a hardness judgment in which the teeth move more than 1 mm. into the test object. With the harder test objects studied, the teeth sink into the test object 0.1 mm. or less, and greater force is required. Then the motion of denture into the test object and toward the mandible or maxilla become similar, and the denture wearer is at a disadvantage compared with the natural dentition case, where axial movement under load is presumably much less.

The denture wearer is at a great disadvantage for detection of very small forces, since the force would normally be spread over several square centimeters of denture supporting area and the pressure would be quite low. The same force on a natural tooth exerts a higher pressure because of the smaller area of the root. The periodontal membrane contains deep pressure nerve endings in close proximity to the tooth which moves under the influence of the force.[7] Both of these conditions help the natural dentition to be more sensitive.

The sensory cues by which $CaCO_3$ is detected in pudding are either vibratory or auditory. The denture is separated from bone by a millimeter or more of tissue, whereas the tooth roots are encased in bone separated by a layer of tissue one third mm. or less in thickness which may be sensitive to vibration. Here again the denture wearer is handicapped probably because of inadequate mechanical "coupling" between the teeth and sensory elements for vibration or sound. It is probable that this handicap has the practical result of diminishing the denture wearers' enjoyment of food. A diet designed for such patients would logically contain foods with striking differences in texture and crispness.

SUMMARY

Judgment of thickness of disks between anterior teeth is similarly acute among denture wearers and persons with a natural dentition. The minimal threshold is 0.18 mm. or 3.3 per cent of disks about 5 mm. thick.

The acuteness of hardness judgments by natural and artificial dentition cases is similar when tested on soft rubber disks between anterior teeth. With hard rubber disks the denture wearers are less sensitive.

The tactile thresholds on biting surfaces of teeth of natural dentition cases ranged from an average of 1 Gm. on anterior teeth to 10 Gm. on molar teeth. The median threshold for eight denture cases was over 125 Gm. at the first premolar position on the denture.

Nine out of ten natural dentition cases were able to detect 2.9 per cent or less $CaCO_3$ in bland pudding, whereas six out of ten denture wearers required over 9 per cent.

In general it appears that the sensory discrimination of denture patients is not impaired when the forces and movements involved are of the magnitude used in mastication. When very small forces or movement are encountered as in judgments of texture, the denture patients seem to possess considerable handicap.

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RELATION OF DENTAL CARIES EXPERIENCE AND GINGIVITIS TO GEOGRAPHIC LOCATION AND SIZE OF COMMUNITY IN MALES 17 TO 21 YEARS OLD (AT THE GREAT LAKES NAVAL TRAINING CENTER)

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EXAMINATION of naval inductees entering the Great Lakes Naval Training Center offered the opportunity to analyze differences in dental caries experience and in gingivitis in men coming from different areas of the United States. Each inductee was examined in a routine fashion for dental decay and periodontal disturbances (using mouth mirror and explorer only). The findings were tabulated as the number of DMF* teeth and tooth surfaces affected per person and the number of P+M+A** gingival units affected per person.

The residence of each man examined was determined as to (1) geographic location and (2) size of community. The distribution of persons examined is tabulated in Tables I and III.

FINDINGS

Dental Caries and Geographic Location.—The data were grouped according to three major geographic areas east of the Mississippi River: Northeast, South and Central (Fig. 1).***

Table I shows that the dental caries experience of men living in the Southern states was significantly less than those coming from the Northeastern area. Men living in the Central states showed a caries experience almost midway between the two and close to the average for the entire group (Fig. 2).

Gingivitis and Geographic Location.—In contrast to the difference in dental caries experience, gingivitis was not significantly different in men coming from the Southern, Northeastern, or Central states (Table II and Fig. 3).

Size of Community.—Analysis of dental caries experience according to the size of the community in which the inductee had resided showed that no significant differences in DMF rates existed in males coming from rural areas, suburban areas, cities or metropolitan areas (Table III). This was also true of gingivitis. The P+M+A rate did not change significantly with the size of the community in which the person had lived (Table IV).

This was a joint investigation in a field of mutual interest by the University of Illinois College of Dentistry and the Dental Department, Administrative Command, United States Naval Training Center, Great Lakes, Illinois, with the approval and support of the Bureau of Medicine and Surgery, Navy Department.

Research grants from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service, supported the phase of the study conducted by the University of Illinois College of Dentistry.

The opinions and assertions of the authors are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

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*D, decayed; M, missing; F, filled teeth or tooth surfaces as outlined by Klein and Palmer.[4]

**P, papillary; M, marginal; A, attached gingival areas as outlined by Massler, Schour and Chopra.[5]

***The number of men coming from the far West was so small and the distribution in area so large that these figures were discarded.

TABLE I

RELATION OF DENTAL CARIES EXPERIENCE TO GEOGRAPHIC LOCATION IN MALES 17 TO 21 YEARS OF AGE EXAMINED AT THE GREAT LAKES NAVAL TRAINING CENTER

AGE IN YEARS	NUMBER OF PERSONS EXAMINED					AVERAGE NUMBER OF DMF TEETH / SURFACE PER PERSON			
	TOTAL GROUP	NORTH-EASTERN STATES	CENTRAL STATES	SOUTH-EASTERN STATES	WESTERN STATES	TOTAL GROUP	NORTH-EASTERN STATES	CENTRAL STATES	SOUTH-EASTERN STATES
17	998	355	281	293	69	9.1 / 19.9	11.0 / 24.6	8.6 / 19.3	7.1 / 14.6
18	619	186	168	200	65	10.1 / 22.6	11.9 / 27.5	9.7 / 21.6	8.6 / 19.2
19	359	90	125	109	35	10.2 / 22.6	12.0 / 27.3	10.7 / 24.3	8.1 / 17.6
20	244	33	112	64	35	11.5 / 25.8	14.2 / 34.1	11.1 / 24.1	9.8 / 22.8
21	148	28	65	33	22	11.3 / 24.2	12.3 / 29.9	11.5 / 24.5	9.6 / 19.8
17-21	2368	692	751	699	226	9.9 / 21.9	11.6 / 26.4	9.8 / 21.8	8.1 / 17.4

Northeast—Conn., Del., Maine, Mass., N. H., N. J., N. Y., Pa., R. I., Vt.
Central—Ill., Ind., Mich., Ohio, Wis.
Southeast—Ala., D. C., Fla., Ga., Ky., Md., Miss., N. C., S. C., Tenn., Va., W. Va.
West—22 states west of the Miss. R. (including Hawaii and Puerto Rico).

DISCUSSION

These findings substantiate previous reports and impressions that dental caries experience was significantly different in men from different geographic areas in the United States.[7] Men living in the southeastern part of this country showed a significantly lower number of DMF teeth than men residing in the Northeast. The Central states seemed to lie between these two extremes.

The reason for regional differences in the caries attack rate is speculative. Analysis of the distribution of fluoride-containing water supplies and oral hygiene habits might serve to elucidate this finding. Mills,[6] Klein and Palmer,[3] East,[2] Klein and Palmer,[4] and Blackerby[1] have suggested that a lower caries attack rate might be related to the greater amount of sunshine experienced by

TABLE II

GINGIVITIS EXPERIENCE IN THREE GEOGRAPHIC AREAS AS DETERMINED IN MALES 17 TO 21 YEARS OF AGE EXAMINED AT THE GREAT LAKES NAVAL TRAINING CENTER

AGE IN YEARS	NUMBER EXAMINED	PERCENTAGE OF PERSONS AFFECTED				AVERAGE NUMBER P + M + Δ GINGIVAL UNITS AFFECTED PER PERSON			
		TOTAL GROUP	NORTH-EASTERN STATES	CENTRAL STATES	SOUTH-EASTERN STATES	TOTAL GROUP	NORTH-EASTERN STATES	CENTRAL STATES	SOUTH-EASTERN STATES
17	998	57.0	55.5	59.8	56.0	4.9	4.4	5.3	5.1
18	619	60.4	61.8	55.4	64.5	5.9	5.3	5.0	7.5
19	359	55.2	53.4	56.8	60.6	5.7	4.6	5.5	6.1
20	244	59.8	66.7	58.0	59.4	6.2	4.8	7.1	5.9
21	148	59.5	67.9	60.0	57.6	5.7	5.4	5.8	7.6
17-21	2368	60.3	58.0	58.1	59.5	5.5	4.7	5.6	6.1

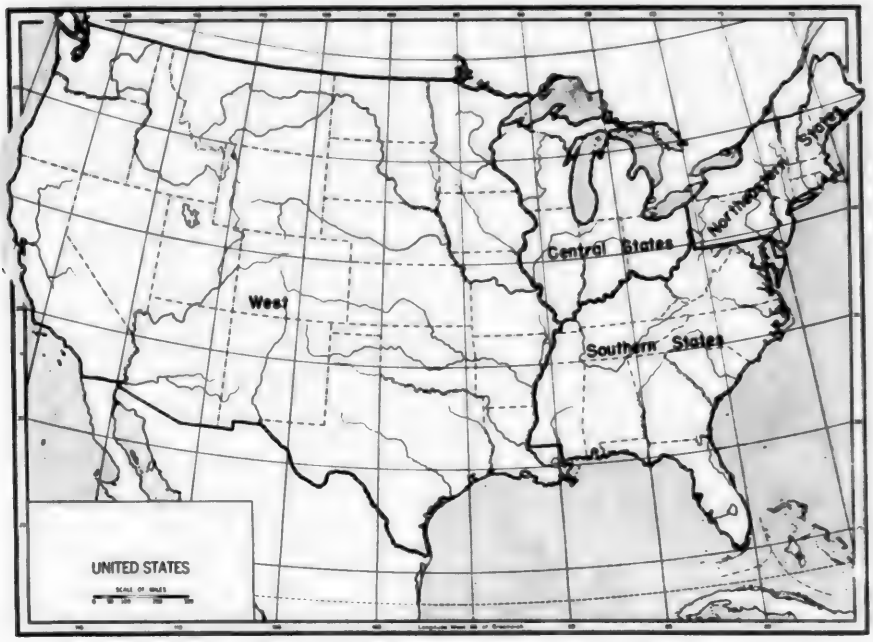

Fig. 1.—Map showing geographic areas from which enlistees were derived.

TABLE III

RELATION OF DENTAL CARIES EXPERIENCE TO SIZE OF COMMUNITY IN MALES 17 TO 21 YEARS OLD EXAMINED AT THE GREAT LAKES NAVAL TRAINING CENTER

AGE IN YEARS	NUMBER OF PERSONS EXAMINED*					AVERAGE NUMBER DMF—TEETH SURFACES—PER PERSON				
	TOTAL GROUP	LESS THAN 2,500	2,500 TO 24,999	25,000 TO 99,999	100,000+	TOTAL GROUP	LESS THAN 2,500	2,500 TO 24,999	25,000 TO 99,999	100,000+
17	994	335	246	160	253	9.1	8.2	9.1	8.8	9.7
						19.9	18.9	19.9	19.1	21.6
18	619	229	197	88	105	10.1	9.4	9.9	11.2	10.9
						22.6	21.1	22.1	24.9	24.9
19	359	130	118	39	72	10.2	9.6	10.2	11.5	10.6
						22.6	21.4	21.9	27.1	23.6
20	242	75	87	38	42	11.5	10.5	12.0	11.9	11.5
						25.8	23.3	27.4	26.8	26.1
21	147	50	42	21	34	11.3	10.3	12.3	11.3	11.5
						24.2	22.0	25.6	26.3	24.6
17-21	2361	819	705	346	506	9.9	9.3	10.1	10.2	10.3
						21.9	20.5	22.2	22.8	23.1

*Arranged according to size of community
Less than 2,500 = Rural communities (farms, villages and small towns)
2,500 to 24,999 = Suburban areas and large towns
25,000 to 99,999 = Cities
100,000 = Metropolis

persons living in the South. This hypothesis should be tested with well-controlled animal experiments. It was very interesting to discover that gingivitis experience appears to be the same in these three areas of the United States.

It was surprising to discover that neither caries experience nor gingivitis was significantly different in persons from rural and metropolitan areas because the mode of living, dietary habits, nutritional status, and oral hygiene have always been assumed to differ markedly in such communities. One had expected a reflection of these in the caries attack rate and gingivitis experience. This study shows that there is apparently no difference in the average amount of caries and gingivitis in persons living in communities of different sizes.

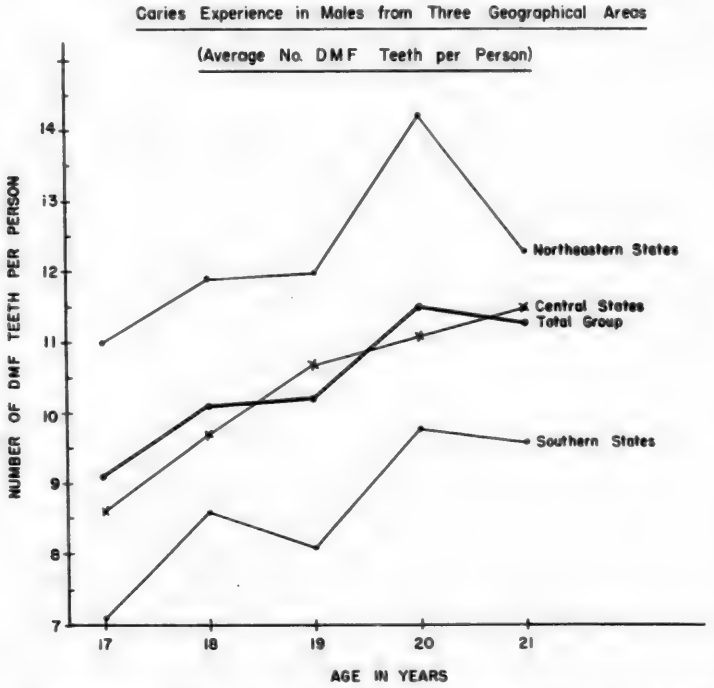

Fig. 2.—Difference in caries experience among 2,368 males, aged 17 to 21 years, coming from 3 geographical areas in the United States. DMF: average number of decayed, missing, and filled tooth surfaces per person.

SUMMARY AND CONCLUSIONS

Dental caries experience and gingivitis were analyzed in 2,368 young adult men 17 to 21 years of age coming from the Northeastern, Central, and Southern states and from communities of different sizes in these areas. It was found that the men coming from the Southern states showed significantly fewer DMF teeth and tooth surfaces than those coming from the Northeast. Persons from the Central states showed a caries experience almost median between the other two areas. The amount and degree of gingivitis was the same in persons from all three areas.

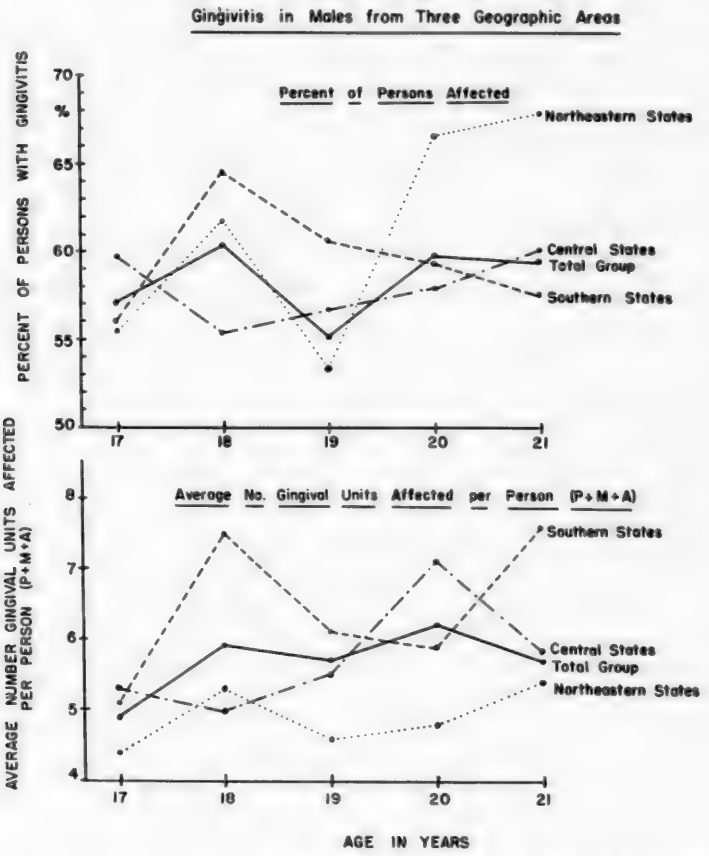

Fig. 3.—Similarity in gingivitis experience among 2,368 males, aged 17 to 21 years, coming from 3 geographical areas in the United States, P+M+A: average number of papillary, marginal, and attached gingival units affected per person.

TABLE IV

RELATION OF GINGIVITIS EXPERIENCE TO COMMUNITY SIZE IN MALES AGES 17 TO 21 YEARS

AGE IN YEARS	PERCENTAGE OF PERSONS AFFECTED BY GINGIVITIS					AVERAGE NUMBER P + M + A GINGIVAL UNITS AFFECTED PER PERSON				
	TOTAL GROUP	LESS THAN 2,500	2,500 TO 24,999	25,000 TO 99,999	100,000+*	TOTAL GROUP	LESS THAN 2,500	2,500 TO 24,999	25,000 TO 99,999	100,000+*
17	60.5	60.0	59.8	57.5	62.1	4.9	5.0	4.5	4.5	5.6
18	63.0	63.8	60.4	55.7	72.4	5.9	7.0	4.9	5.3	5.9
19	58.2	57.7	52.5	76.9	56.9	5.7	6.2	5.1	6.3	5.2
20	61.2	61.4	58.6	60.5	61.9	6.3	7.0	6.5	5.6	5.1
21	60.5	60.0	59.5	61.9	61.8	5.7	6.6	5.5	7.1	3.8
17-21	60.8	61.1	58.3	59.8	63.2	5.5	6.1	5.0	5.2	5.4

*Population of community residence (see footnote to Table III).

The number of carious (DMF) teeth or tooth surfaces and the number of inflamed gingival units (P+M+A) was not significantly different in men coming from rural, suburban, city or metropolitan communities.

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RELATION OF DENTAL CARIES EXPERIENCE AND GINGIVITIS TO CIGARETTE SMOKING IN MALES 17 TO 21 YEARS OLD (AT THE GREAT LAKES NAVAL TRAINING CENTER)

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THE question has often been raised as to whether there is any relationship between smoking (or chewing) tobacco and dental caries experience. A similar question might be raised as to the relation between smoking and gingivitis.

METHODS AND MATERIALS

This study was based upon the examination of 2,577 naval enlistees at the Great Lakes Naval Training Center. They represented a fair cross section of the youth living east of the Mississippi River. All were adult white males between the ages of 17 to 21 years. The number of DMF* teeth and tooth surfaces and the number of inflamed gingival units (PMA)† in each person were calculated as previously described.[3] Each person was asked to state the total number of cigarettes (or other tobacco) which he consumed per day.

FINDINGS

Number of Cigarettes Smoked per Day.—Fig. 1 shows that the frequency distribution in the number of cigarettes smoked per day fell sharply into four groups: those who consumed no tobacco and those who smoked 5, 10, 15, or 20 cigarettes per day. Since these were young adult males, 17 to 21 years of age, the length of time each person had been smoking varied from one to six years according to age.

Relation of Dental Caries Experience to Cigarette Smoking.—Table I shows that at the ages of 17 and 18, those who did not smoke, or smoked only 5 cigarettes per day had slightly less caries than those who smoked 15 or 20+ cigarettes per day. The difference was statistically significant. At the ages of 19 and 20 years the difference was large and very significant. Those who smoked less than 6 cigarettes per day showed a significantly lower number of DMF teeth and tooth surfaces than those who smoked 15 or more cigarettes per day (Table I and Fig. 2). This difference was marked after 19 years of age presumably because of the longer time of smoking. The difference became less at the age of 21 because of the small number of persons examined.

The opinions and assertions of the authors are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large. Received for publication, Sept. 10, 1951; Revised, Dec. 6, 1951.

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Research grants from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service, supported the phase of the study conducted by the University of Illinois College of Dentistry.

*D, decayed; M, missing; F, filled teeth or tooth surfaces (Klein and Palmer[1]).

†P, papillary; M, marginal; A, attached gingival units affected (Massler, Schour, and Chopra[2]).

TABLE I

RELATION OF CIGARETTE SMOKING TO DENTAL CARIES EXPERIENCE (AVERAGE NUMBER DMF TEETH AND SURFACES) AND GINGIVITIS (AVERAGE NUMBER PMA GINGIVAL UNITS AFFECTED)

NUMBER PERSONS EXAMINED							AVERAGE NUMBER DMF* TEETH / SURFACES PER PERSON						AVERAGE NUMBER PMA† GINGIVAL UNITS AFFECTED PER PERSON					
AGE IN YEARS	TOTAL GROUP	AVERAGED NUMBER CIGARETTES CONSUMED PER DAY					TOTAL GROUP	AVERAGE NUMBER CIGARETTES CONSUMED PER DAY					TOTAL GROUP	AVERAGE NUMBER CIGARETTES CONSUMED PER DAY				
		0	5	10	15	20+		0	5	10	15	20+		0	5	10	15	20+
17	1108	236	112	305	119	336	9.0 19.6	8.3 18.0	8.6 19.4	9.4 20.5	8.5 18.5	9.45 20.4	5.1	5.4	5.1	4.7	5.6	5.1
18	677	184	58	172	74	180	10.0 22.4	10.2 21.4	9.7 22.0	10.1 22.9	9.7 20.3	10.1 23.7	5.9	5.4	5.8	5.2	6.5	6.8
19	385	112	30	72	45	126	10.0 22.4	9.7 21.5	8.8 19.5	10.2 21.3	9.7 22.6	10.5 24.4	5.5	5.5	6.2	6.1	6.3	4.7
20	253	72	25	43	30	83	11.4 25.4	10.1 21.6	9.5 21.5	11.6 26.1	13.8 30.2	12.3 28.8	6.15	6.9	6.0	6.1	5.6	5.8
21	154	40	10	28	17	59	11.3 24.4	11.8 24.7	11.3 24.5	10.1 20.8	11.2 25.0	11.5 25.2	6.0	4.6	4.8	6.5	8.3	6.3
17-21	2577 (100%)	644 (25%)	235 (8.5%)	620 (24%)	285 (11.2%)	793 (30.8%)	9.8 21.7	9.5 20.4	9.1 20.5	9.8 21.7	9.75 21.2	10.2 23.0	5.55	5.6	5.5	5.2	6.1	5.6

*DMF, Decayed, missing and filled teeth or tooth surfaces.
†PMA, Number of papillary, marginal, and attached gingival units affected by inflammation.

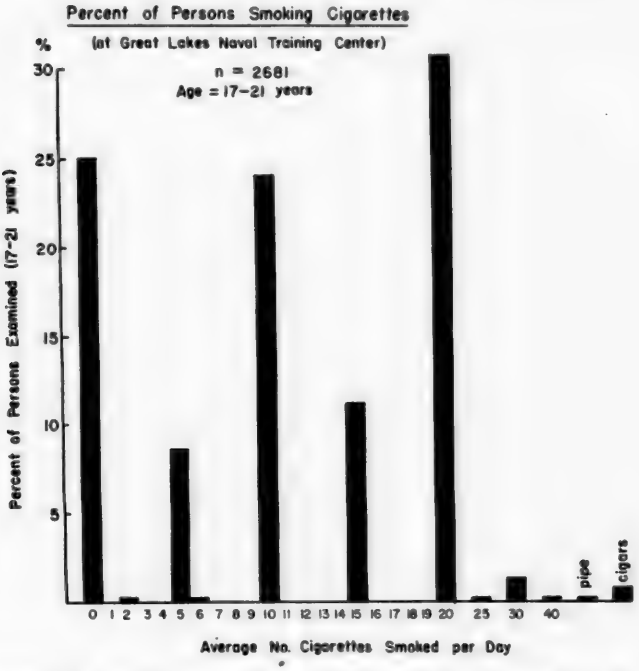
Percent of Persons Smoking Cigarettes
(at Great Lakes Naval Training Center)
n = 2681
Age = 17-21 years
%
Percent of Persons Examined (17-21 years)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 25 30 40
pipe
cigars
Average No. Cigarettes Smoked per Day

Fig. 1.—Distribution of persons consuming tobacco in 2,681 males, ages 17 to 21 years, examined at the Great Lakes Naval Training Center.

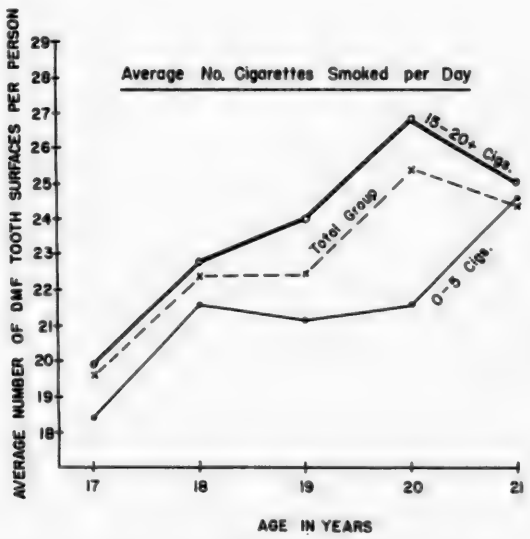

Fig. 2.—Difference in caries experience between persons smoking only occasionally or not at all and those consuming more than 15 cigarettes per day.

Gingivitis.—The number of inflamed gingival areas was not significantly correlated with different degrees of smoking (Table I). There did not appear to be any relationship between cigarette smoking and gingivitis in this group.

DISCUSSION

The finding that dental caries experience was significantly less in those who did not smoke or smoked seldom was unexpected in view of the common supposition that those who smoke (or chew) tobacco heavily show less caries. Schmidt[6] reported that an increase in tobacco smoking was followed by a decrease in caries rate. In this group a caries-inhibiting effect of tobacco was not apparent. The reverse was evident. However, the fact that these men had been smoking for a relatively short time should be taken into account.

It should be pointed out that the relation of smoking to caries experience as observed in this study may be merely coincidental. Whether any causal relationship exists, either direct or indirect, needs to be tested by controlled experimental observations.

The gingivitis rate was not significantly different in smokers and nonsmokers. Pindborg[4, 5] examined a large group of Danish seamen and similarly found that the number of persons with simple marginal gingivitis was not different in smokers or nonsmokers although the incidence of ulceromembranous gingivitis and calculus was significantly greater in the group of smokers. In our group, the incidence of ulceromembranous gingivitis was very low (20 mild cases in 3,880 enlistees). In the group with ulceromembranous gingivitis, 13 men smoked more than 16 cigarettes per day, 6 men smoked from 6 to 15 cigarettes per day, while only one did not smoke at all. This confirms Pindborg's observations.

SUMMARY AND CONCLUSIONS

The relation of cigarette smoking to caries experience and gingivitis was investigated in 2,577 naval enlistees, 17 to 21 years of age. It was found that those who smoked more than an average of 15 cigarettes per day had a significantly greater number of decayed, missing, and filled teeth and tooth surfaces than those who smoked less than 6 cigarettes per day. On the other hand, the amount of simple gingivitis was not related to the number of cigarettes smoked per day.

We wish to express our appreciation to Captain C. W. Schantz, DC USN, Dental Officer, for his aid and cooperation; to Dr. Jack Zwemer, who supervised the statistical tabulations by means of I.B.M. cards; to LCDR E. T. Guay, DC USN, for the caries examinations; and to K. B. Bass, M.S., and W. R. Martin, DN USN, for their active participation in the survey of the enlistees.

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TESTING THE UNIFORMITY OF THE P-M-A INDEX AS A MEASUREMENT OF PERIODONTAL DISEASE

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THE P-M-A Index proposed by Massler and Schour* and used in many studies of periodontal disease, is said to combine "simplicity, speed, accuracy, quantitativeness, and reproducibility." Its proponents point out that the measurement records the extent of the disease by counting the number of gingival units affected. They also note that, since inflammatory lesions of the gingiva tend to begin in the papillary gingiva, to spread from that area into the marginal gingiva and, in severe gingivitis, to affect the attached gingiva (periodontitis), the location of the abnormality is an additional index of the severity of the disease.

Essentially, the measurement depends upon the differentiation between normal and inflamed gingiva. Extreme inflammatory reactions are easily recognizable but the differentiation of mildly inflamed gingiva is not as clear cut. Although the index is advocated for all ages, its greatest area of usefulness would seem to be directed toward children because inflammatory changes of the gingiva are most characteristic of the younger ages, and because children are more likely to have most of their teeth present. In older age groups, uncomplicated gingival inflammation is comparatively less frequent while atrophic and noninflammatory periodontal reactions, such as gingivosis, periodontosis and recession, occur more often. As the number of teeth present in the mouth decreases with age, the measurement decreases in significance. The number of papillae or marginal gingiva or attachments can be no larger than the number of teeth in the mouth and ratios based upon a few teeth can be very misleading.

Because of the need for better objective measurements of periodontal disease, and, despite these theoretical limitations of the P-M-A Index, this measurement was applied to an adult group to determine if different examiners are likely to obtain the same findings. The dental staff at the Brooklyn Veterans Hospital carefully reviewed the criteria of the measurement and a uniform examination and recording technique was outlined. *P* was defined as "any degree of inflammation of the interdental papilla mesial to the tooth"; *M* was defined as "any degree of inflammation of the marginal gingiva on the facial aspect"; and *A* was defined as "any disturbance of attachment as indicated by any degree of recession of the marginal gingiva from normal contour." The examinations were performed by dentists and were recorded by trained dental assistants. After several weeks of experience with the technique, a test was planned and executed.

Within two or three days after their admission to this hospital, all patients who are not too ill for such an experience, receive a dental and oral examination, including a full mouth series of intraoral x-rays. On a specified day, ten patients who had eight or more teeth, were selected and were examined by all

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*Massler, M., Schour, M., and Chopra, B.: Occurrence of Gingivitis in Suburban Chicago Children, *J. Periodont.* 21: 146, 1950.

TABLE I

THE NUMBER OF PERIODONTAL LESIONS IN TEN PATIENTS AS DETERMINED BY EIGHT DENTISTS

	PATIENT																														
	A			B			C			D			E			F			G			H			I			J		
DENTIST	P*	M*	A*	P	M	A	P	M	A	P	M	A	P	M	A	P	M	A	P	M	A	P	M	A	P	M	A	P	M	A	P	M	A
1	14	14	14	14	16	0	15	16	16	0	0	0	0	0	0	0	0	0	5	3	0	18	15	12	2	0	0	18	18	18
2	12	3	4	18	16	6	16	16	16	5	5	0	5	0	0	6	3	0	20	5	1	3	2	1	9	2	0	19	18	6
3	3	4	7	9	7	10	11	13	14	0	0	0	1	3	0	0	1	0	10	8	0	4	5	1	1	1	0	19	20	20
4	7	6	11	14	13	16	15	11	18	4	6	0	1	0	0	4	3	0	13	8	0	3	3	3	5	3	1	16	17	17
5	3	2	14	8	11	16	16	15	15	0	0	0	2	1	0	0	0	0				0	0	2	1	0	0	19	5	6
6	6	4	5	8	8	4	11	7	9	0	1	1	2	1	1	1	2	0	11	9	1	1	2	0	1	1	0	15	15	4
7	4	6	10	9	17	12	10	9	19	6	6	3	4	4	2				13	14	9	9	9	7	1	2	2	12	11	11
8	6	6	13	11	18	12	16	17	17	7	6	7	2		0				21	19	19				2	9	0	17	15	12
Total	55	45	78	91	106	76	110	104	124	22	24	11	17	9	3	11	9	3	93	66	30	38	36	26	22	18	3	135	119	94

*P, Any degree of inflammation of the interdental papilla mesial to the tooth.
M, Any degree of inflammation of the marginal gingiva on the facial aspect.
A, Any disturbance of attachment as indicated by any degree of recession of the marginal gingiva from normal contour.

of the dentists, including a periodontist. Lighting and instruments remained the same for each patient and the P-M-A Index was recorded for each patient by the same dental assistant.

The findings varied widely from patient to patient, but the variations between dentists on examinations of the same patient (Table I) were unexpectedly wide. Theoretically, all of the examiners should obtain the same readings on the same patient. However, differences of concentration, interest, training and technique may all influence the number of defects observed.

Since any series of measurements, however accurate the measuring rod, may vary by chance, the differences observed by this group of dentists might also fall within reasonable limits of chance variation. The question asked about these observations was; what is the probability that such a diversity of findings could ocur by chance alone?* Table II lists the probability that a diversity of findings as wide as was found by this group of dentists might be expected to

TABLE II. THE AVERAGE NUMBER OF PERIODONTAL LESIONS FOUND BY EIGHT EXAMINERS WHO EXAMINED THE SAME PATIENTS, AND THE PROBABILITY THAT THE VARIATION OF OBSERVATIONS COULD HAVE BEEN DUE TO CHANCE

PATIENT	DEGREES OF FREEDOM (N)	NUMBER OF PAPILLAE INFLAMED (P)			NUMBER OF MARGINAL GINGIVAE INFLAMED (M)			NUMBER OF GINGIVAE RECEDED (A)		
		MEAN	χ^2	PROBABILITY	MEAN	χ^2	PROBABILITY	MEAN	χ^2	PROBABILITY
A	7	6.9	9.65	.22	5.6	16.60	.02*	9.7	11.48	.12
B	7	11.4	7.06	.43	13.2	9.37	.20	9.5	24.22	.001*
C	7	13.8	3.53	.85	13.0	5.06	.66	15.5	4.79	.70
D	7	2.8	26.78	<.001*	3.0	20.73	.005*	1.3	33.79	<.0005*
E	7	2.1	9.05	.25	1.1	15.73	.03*	0.37	10.42	.15
F	7	2.8	20.58	.005*	2.2	27.04	<.001*	0.37	10.42	.15
G	7	16.9	2.63	.90	14.8	9.61	.22	11.7	21.37	.004
H	5	1.8	17.93	.003*	1.5	6.33	.30			
I	6	13.3	13.13	.04*	9.4	18.37	.006*	4.3	73.80	<.0005*
J	6	5.4	44.78	<.0005	5.1	31.99	<.0005*	3.7	30.10	<.0005*

*Variation of examinations as great as these, might be expected to occur by chance alone very infrequently.

occur by chance alone. Accepting the conventional 5 per cent level of significance, it will be noted that four out of the ten papillae series are improbable of chance occurrence. Similarly, of the marginal inflammation series, five are improbable, and of the attachment series, five are improbable. No pattern of agreement could be deduced as between high or low average findings and the probability of agreement between examiners.

CONCLUSIONS

The P-M-A Index as a measurement of periodontal disease of adults, suffers from serious theoretical deficiencies. A test of the measurement demonstrated that the findings of eight dentists upon examinations of the same group of adult patients, tend to be widely divergent.

The fact that different dentists do not tend to agree upon the measurement does not preclude its use by a single observer, but any comparison of observations made by different dentists must be made with extreme caution.

*The Chi square test is particularly suited to measure the concordance between these observations and the most probable expected finding, the mean.

THE EFFECT OF TOBACCO SMOKE UPON SOME BACTERIA

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ACCORDING to Hobson,[5] 21 million smokers over the age of sixteen consume weekly almost 4 million pounds of tobacco in Great Britain. Apart from psychologic factors the following organ systems have been cited as influenced by tobacco: the cardiovascular system, the oral and gastric mucosa, and the pulmonary system. The possible influence of tobacco smoke on oral bacteria has been investigated,[1, 7, 10] as well as the relationship between the incidence of Vincent's infection and gingivitis and tobacco,[6, 11, 12, 15] and leukoplakia[3, 4, 14, 17, 18] and tobacco.

The object of the present study was to determine the effects of tobacco smoke upon some bacteria and on those normally occurring in the mouth. The supposed curative effects of tobacco smoke have long attracted attention; in fact, smoking was at one time recommended for its healing powers. According to de Kruif[8] Spallanzini first observed, in the middle of the 18th century, the adverse influence of tobacco smoke on microbes.

W. D. Miller[10] and many others tested the sensitivity of bacteria to tobacco smoke in vitro and in vivo. They found, and this was confirmed by Appleton,[1] that tobacco smoke is far more bacteriostatic and bactericidal in vitro than in the mouth. On agar, in broth, and on paper or linen strips most pathogenic bacteria are killed within 5 to 60 minutes. Arnold[2] remarked that "tobacco smoke as drawn into the mouth is very probably detrimental to the growth of some pathogenic organisms, but not more, indeed rather less so than smoke from other sources." Puntoni[13] found that *B. typhosum* on infected rabbit gut survived the smoke of two cigars for more than 60 minutes. Meuly[9] reported that *C. diphtheriae* on rabbit gingiva was still viable after two hours' exposure to tobacco smoke. Twort and Bakker[19] reported that the germicidal action of an aerosol was completely eliminated by cigarette smoke.

Appleton[1] stated that the smoking of a cigarette temporarily reduced the bacterial count in the mouth which quickly returned to normal. The evidence obtained by many workers shows that tobacco smoke possesses bacteriostatic and bactericidal properties in vitro but that in the mouth it is far less potent and apparently not bactericidal.

PRESENT INVESTIGATION

Two series of experiments were performed to obtain further information. In the first, four agar slopes were inoculated with (1) *Staph. aureus* (Oxford strain No. 6511 Nat. Collection of Type Cultures), (2) *E. coli* (86 N.C.T.C.), (3) *B. subtilis* (3610), and (4) *L. acidophilus odontolyticus* (1406). After an eight-hour incubation the cotton plugs in the tubes were exchanged for sterilised rubber stoppers each of which was perforated by a sterile glass cigarette holder

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*Research Department, International Serum Company.

and a sterile glass mouthpiece. One cigarette was then smoked through each tube. This took five to ten minutes. During this time, the agar cultures became covered with a thin oily film. Both the colonies on the agar and the water condensing at the bottom of the tubes were tested for bacterial growth immediately after smoking, 24 hours later and 48 hours later. Table I shows that only *E. coli* was immediately affected by the smoke. After 24 hours the condensing fluid in the *Staph. aureus, E. coli* and *L. acidophilus odontolyticus* was sterile and the *E. coli* colonies on agar were no longer viable. After 48 hours *B. subtilis* showed diminished growth but *L. acidophilus odontolyticus* growing in the condensing fluid had recovered.

TABLE I

BACTERIA TESTED	IMMEDIATELY AFTER ONE CIGARETTE	AFTER 24 HOURS INCUBATION	AFTER 48 HOURS INCUBATION
Staph. aureus Water of Condensation	+++	–	–
Staph. aureus Agar	+++	+++	+++
B. coli Water of Condensation	+++	–	–
B. coli Agar	++	–	–
B. subtilis Water of Condensation	+++	+++	+
B. subtilis Agar	+++	+++	++
L. acidophilus odontolyt. Water of Condensation	+++	–	++
L. acidophilus odontolyt. Agar	+++	+++	+++

+++ indicate normal growth
++, + indicate less growth
– indicate no growth

The second series of experiments approached the problem from a different angle. Saliva agar plates were prepared by washing out the mouth with normal saline. The resulting fluid was then passed through a Seitz filter and mixed with liquid agar. Having been tested for sterility these plates were then inoculated with (1) *P. pyocyanea* (1999), (2) *Chrom. prodig.* (1377), (3) *B. subtilis* (3610), (4) *E. coli* (86), (5) *Staph. aureus* (6511), (6 & 7) two different strains of *L. acidophilus odontolyticus* (strains "S" and "Cr"), and (8) saliva. These plates and a plain agar plate served as controls. A cigar was smoked, the smoke being retained in the mouth as long as possible. While retaining the smoke in the mouth, the latter was washed with sterile saline and the resulting "tobacco smoke—saliva—saline" was then emptied into a beaker, and passed through a Seitz filter. The sterile filtrate was then mixed with liquid agar, poured into Petri dishes and inoculated with the same bacterial strains mentioned before.

No difference in growth could be detected between the cultures grown on plain agar and those grown on "tobacco—saliva—agar." A remarkable difference in pigment production was however noticeable. *P. pyocyanea* and *Chrom. prodigiosum* formed hardly any pigment on plain agar; on saliva agar the

colonies of *P. pyocyanea* were slightly pink, while those of *Chrom. prodigiosum* were typically red and grew luxuriantly at 37° C. Observed in filtered ultra violet light colonies of *P. pyocyanea* were yellow-green and fluorescent, those of *Chrom. prodigiosum* appeared as brown-red with a grey circumference.

On "tobacco-saliva-agar" colonies of *P. pyocyanea* were very strongly yellow and fluorescent whereas those of *Chrom. prodigiosum* were of a brilliant purple with a dark grey circumference. The remaining strains showed up normally under filtered u.v. light. The pH of the normal agar was 7.0, that of the saliva-agar 7.2 and that of the "tobacco-saliva-agar" 7.4.

SUMMARY

Experiments have confirmed both the bacteriostatic and the bactericidal effect of tobacco smoke on certain organisms in vitro, these effects being more marked in a liquid medium than on solid agar. Bacterial colonies grow as freely on agar mixed with saliva treated with tobacco smoke as on plates containing only normal saliva. Two of the colonies grown on "tobacco-smoke-saliva" showed distinctive pigmentation.

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ALKALINE PHOSPHATASE ACTIVITY OF HEALING GINGIVAL WOUNDS IN THE RAT

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THE enzyme, alkaline phosphatase, has a wide distribution in normal animal tissues[1, 2] and is believed to subserve a number of important metabolic functions.[3, 4, 5] The activity of this enzyme in the buccal epithelium has been shown to fluctuate in response to changes in blood level of estrogenic hormone during the normal estrous cycle in the rat.[6] Normal gingival epithelium, however, exhibits a constantly low level of alkaline phosphatase activity in man and several animal species.[6-10]

It has been demonstrated that maximal activity of epithelial alkaline phosphatase coincides with peak proliferative or metabolic activity of the cells.[11] The distribution of zones of enzymatic activity in cornifying membranes suggests a relationship to the process of keratinization.[6, 12] It has been postulated that the level of this enzyme necessary to maintain the keratinization of the gingiva in the presence of normal attrition may be too low for histochemical demonstration.[6]

The present experiments were undertaken in order to study any changes from the normally low epithelial alkaline phosphatase activity which might occur in gingivae healing from surgical wounds.

METHODS

Forty-six normal albino rats were used in these experiments; 24 females and 22 males. All animals were anesthetized with Nembutal. The interdental papilla of the maxillary incisor teeth was first swabbed with a 1:2500 solution of Metaphen. An incision was then made in the midsagittal plane for a distance of about 3 mm., stopping just short of the free margin. Gentle pressure was applied until hemorrhage was controlled. In a few cases, two parallel incisions were made about 1 mm. apart, with one of the incisions somewhat shallower than the other.

The animals were sacrificed in 3 groups, each group comprising approximately equal numbers of males and females. The first group of 24 animals was killed 24 hours after infliction of the wound. The second and third groups each contained 11 animals and were sacrificed 48 and 72 hours, respectively, after the gingivae were incised. The reason for the larger group representing the earliest observed stage of healing was the frequent massive acute inflammatory reaction during the first 24 hours which tended to obscure evidences of early re-epithelialization. By 48 hours, the inflammation had subsided in most instances so that the architecture of the lesion could be seen.

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The animals were killed by decapitation and the incised papillae dissected free. These were fixed in cold 80 per cent ethyl alcohol and sectioned in paraffin, perpendicular to the plane of the incision, at 7 microns. Sections were incubated in glycerophosphate buffered to a pH of 9.4 and at a temperature of 37° C. for periods of ½, 1, 2, and 24 hours, according to the method of Gomori[1] (modified by the addition of 0.1 M $MgSO_4$ to the incubating medium). Control sections were incubated in a medium lacking in the glycerophosphate substrate. Other sections of the wounded area were stained with Harris hematoxylin and eosin.

Normal control tissues were obtained from untreated animals as well as from normal areas distal to the region of the incision but on the injured papilla. Normal control sections were also stained by the Gomori method and by hematoxylin and eosin. No histological or histochemical differences were observed in the gingival tissues from males and females, normal or experimental.

RESULTS

Gomori preparations of normal gingiva of the rat show a faint but uniform background coloration in the epithelium and in cells and fibers of the connective tissue. This background staining increases but slightly with prolonged incubation. Superimposed on the background is a darker color resulting from increased deposition of cobalt sulfide in the superficial band of keratin, in the keratohyalin granules, in the nuclei and especially the nucleoli and nuclear membranes of more basally placed epithelial cells, in some of the small superficial blood vessels, and in the nuclei of scattered connective tissue cells. These zones of augmented precipitate show further blackening upon prolonged incubation with substrate and apparently represent the principal sites of alkaline phosphatase activity in this tissue. In sections of some papillae, there are random areas of lamina propria which are darker than the surrounding tissue. Here a heavier precipitation has occurred in larger numbers of cells, fibers and blood vessels.

In general, there is evidence of higher enzymatic activity in the deeper layers of connective tissue than in the more superficial. Also the region of the epithelial attachment (Fig. 1) exhibits evidence of considerably higher activity both in the stroma and in the epithelium than does the gingiva attached to the alveolar ridge (Fig. 2).

Gingivae of the rat healing from an experimentally inflicted wound exhibit a greatly enhanced deposition of cobalt sulfide indicative of a marked increase in alkaline phosphatase activity. This apparently augmented enzymatic activity is centered in the lesion itself but may extend for a short distance on either side. The zone of greatest precipitation is the superficial epithelium beyond the layer of granular cells (Fig. 4). Routine histologic preparations show this as a thickened band of keratin containing some pyknotic nuclei (Figs. 3, 5, 7, and 9). Extending basally, and in the immediate vicinity of the epithelial cicatrix, are scattered, deep-staining epithelial cells. The remainder of the epithelium in the vicinity gives evidence of some increase in enzymatic activity, especially of the nuclei.

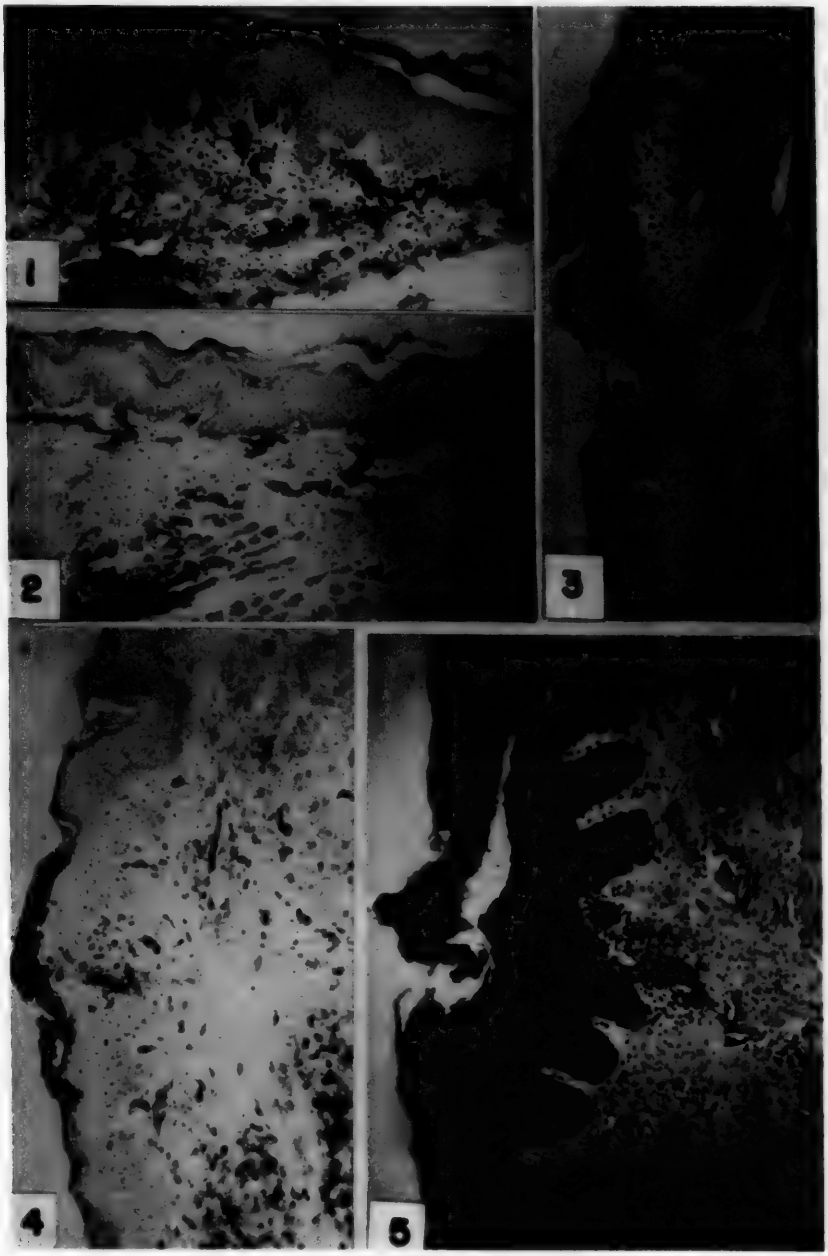

Fig. 1.—Normal free gingiva. Phosphatase preparation, incubated 24 hours. Orig. mag. ×120.

Fig. 2.—Normal attached gingiva. Phosphatase preparation, incubated 24 hours. Orig. mag. ×120.

Fig. 3.—Incised gingiva—24 hours' healing. H.&E. Orig. mag. ×120.

Fig. 4.—Same tissue as Fig. 3. Phosphatase preparation, incubated ½ hour. Orig. mag. ×120.

Fig. 5.—Incised gingiva—48 hours' healing. H.&E. The tangential separation of the epithelium extending toward the top of the figure is a sectioning artifact. Orig. mag. ×120.

With a longer period of healing, the prickle cell layer may give evidence of increased phosphatase activity in the form of a lattice-work distribution of cobalt sulfide at the periphery of the cells and in the intercellular space (Figs. 6 and 8).

Healing of the gingival epithelium appears to involve an approximation of the cut edges and rearrangement of the cells. Alkaline phosphatase activity appears to wane first in the deeper layers and persist latest in the thickened keratin "scar" (Figs. 9 and 10).

The lamina propria, like the epithelium, gives evidence of heightened alkaline phosphatase activity not only in the immediate vicinity of the lesion but for some distance on each side. The site of the incision is marked by an organizing fibrin clot containing a finely dispersed cobalt sulfide precipitate, slightly darker than that of the adjacent connective tissue fibers. In the meshes are a few small cells or nuclei intensely blackened. Routine preparations indicate invasion of the clot by capillary endothelium, which does not, however, give as intense a phosphatase reaction as do the superficially located vessels of the normal areas. Around the periphery of the clot are numerous scattered cells staining intensely for phosphatase. These appear to be macrophages with, in some instances, granules of cobalt sulfide visible in their cytoplasm. Nuclei of fibroblasts are faintly colored by the reaction, and in the vicinity, especially at the periphery, of the lesion these exhibit a deposit of cobalt sulfide particles adherent to or extending a short distance from the nuclear membrane.

DISCUSSION

Normal gingival epithelium of the rat and a number of other species including man have been shown to be relatively devoid of alkaline glycerophosphatase,[6-10] although Cabrini and Carranza[9] have obtained a positive epithelial phosphatase reaction using nucleic acid as substrate. Ring and Levy[6] reported the absence of gingival epithelial phosphatase in the rat even when the adjacent buccal epithelium showed histochemical evidence of a high degree of enzymatic activity.

In stratified squamous membranes which do exhibit phosphatase activity it has been postulated on the basis of distribution that the activity of the enzyme may be related to the process of keratinization.[6, 12] Epithelial cicatrization of cutaneous wounds healing by granulation involves keratinization which proceeds from the margins.[13] It can be seen from the present study that in healing gingival wounds it is the keratinizing area of the epithelium in and adjacent to the incision which shows the most intense phosphatase picture (Figs. 4, 6, and 8) and in which enzymatic activity remains elevated for the longest period (Fig. 10). In no instance is this activity associated with proliferating cells.

Wound healing in the rat gingivae appears to involve: (1) the closing of the wound by a fibrin clot, (2) the organization of the clot by capillary buds, (3) the approximation of the incised epithelial surfaces (possibly by contraction of the granulation tissue organizing the defect). Alkaline phosphatase appears to be involved in: (1) the growth and function of the outgrowing capillaries, (2) the activity of fibroblasts in connection with development of connective

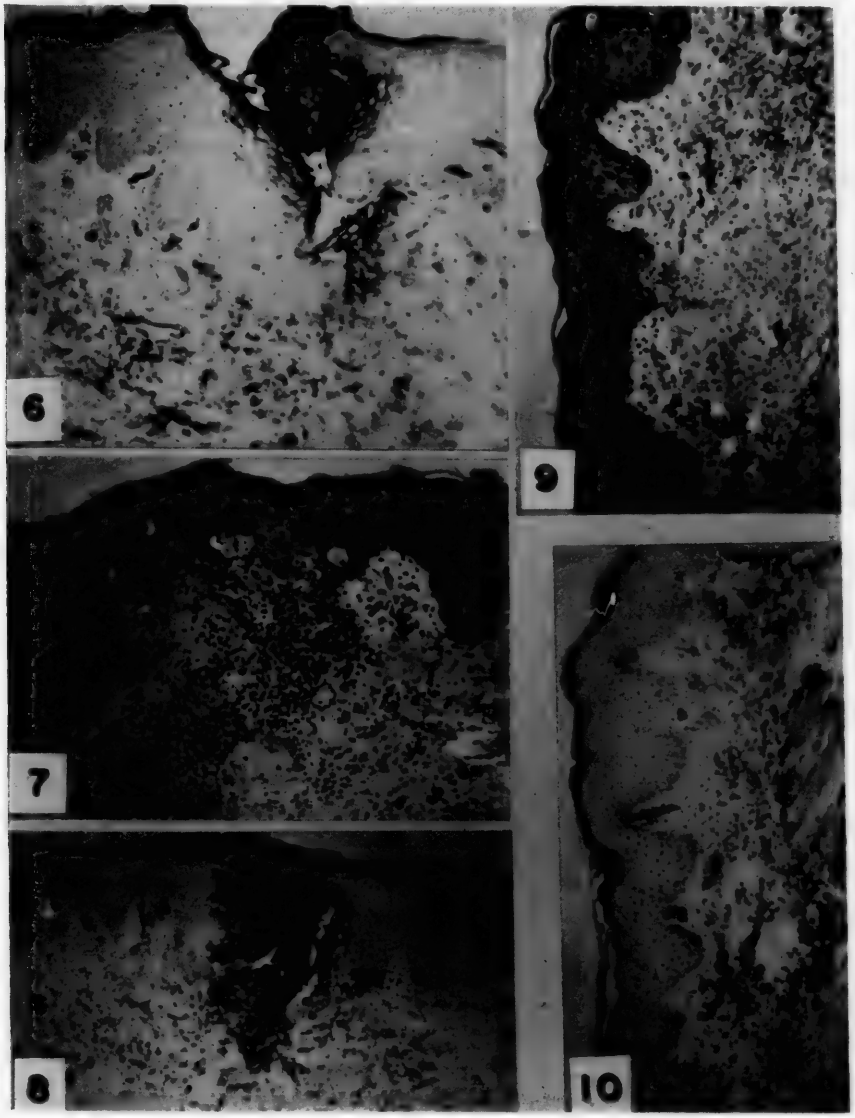

Fig. 6.—Same tissue as Fig. 5. Phosphatase preparation, incubated ½ hour. Only a minute area in the center of the incision remains open as in this section. Fig. 5 thus represents the adjacent, already healed margin of the lesion. Orig. mag. ×120.

Fig. 7.—Incised gingiva—72 hours' healing. H.&E. Orig. mag. ×120.

Fig. 8.—Same tissue as Fig. 7. Phosphatase preparation, incubated ½ hour. Orig. mag. ×120.

Fig. 9.—Incised gingiva. Complete healing of shallow wound in 24 hours. H.&E. Orig. mag. ×120.

Fig. 10.—Same tissue as Fig. 9. Phosphatase preparation, incubated 24 hours. Orig. mag. ×120.

tissue fibers, (3) the activity of macrophages in performing their scavenger function, and (4) keratinization proceeding from the margins of the epithelial cicatrix.

It is worthy of note that the apparent distribution of maximal phosphatase activity in the intercellular spaces of active areas of gingival epithelium healing from an experimental wound has its counterpart in phosphatase active areas of buccal epithelium[6] and in the cornifying vaginal epithelium[12] of the rat. A similar inter- and pericellular distribution of sudanophil material (lipid) and glycogen has been reported in normal human gingival epithelium,[10] and periodic acid-Schiff reactive, saliva resistant material (glycoprotein) is similarly located in human and monkey gingiva, esophagus, skin, vagina and other tissues.[14]

SUMMARY

Experimental wounds of the gingiva exhibit evidence of a marked augmentation of alkaline phosphatase activity in the outer layers of epithelium adjacent to the incision and in and surrounding the organizing fibrin clot in the lamina propria. The increased enzymatic activity in the region of the healing wound appears to be associated with: (1) the growth and function of outgrowing capillaries, (2) the activity of fibroblasts in connection with the development of connective tissue fibers, (3) the activity of macrophages within and around the lesion, and (4) keratinization proceeding from the margins of the epithelial cicatrix.

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THE DENTAL TREATMENT NEEDS OF NAVAL PERSONNEL AS DERIVED FROM 1570 CASES

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THE purpose of this study is to determine the amount and type of dental care needed by regular Navy and Naval Reserve personnel during various stages of their naval careers. Since the answer must be in the form of a prediction, the basis of the prediction must come from the facts collected from a representative group of men now in the Navy. The group selected for this study consisted of the crew of a heavy cruiser which was recommissioned in January of 1951. The crew was drawn from regular and reserve sources during the present build up of the armed forces. An opportunity was thereby presented for evaluating the dental condition of the entire crew by groups. This data forms the basis of this study.

It is felt that studies of this type are needed to evaluate the present dental needs of the armed forces as a whole. They will aid in forecasting future needs thereby aiding in allocating treatment facilities and in testing the validity of existing dental requirements for entry into the various branches of the armed forces. It is hoped that this study will be evaluated and others of similar design be initiated to further define the problem.

METHODS

Each officer and enlisted man was given a thorough oral examination. After observing the lips, two mouth mirrors were used as retractors and the mucous membrane of the lips, cheeks, palate, sublingual, and submaxillary regions were inspected for the presence of growths, tumors, hard nodes, leukoplakia, and evidences of specific infections. The gingivae were examined for fistulous openings, disturbances of the interproximal spaces, recessions or hypertrophies, and presence of pockets and periodontal infections. Each individual was asked if he had ever had any disease of the soft tissue. If the answer was affirmative, a check was made for a possible chronic condition. The examination of the teeth was done by means of sharp explorers, mouth mirrors, and compressed air. Limited time prohibited a prophylaxis prior to the examination. However, unclean mouths were given more time on examination. Due to a shortage of bitewing film, two $1\frac{1}{16}$ by $2\frac{1}{8}$ posterior bitewing radiographs were exposed on every third man only. Periapical films were exposed on all pulpless teeth, teeth with pulps of doubtful vitality, teeth with evidence or histories of fistulae, area of missing teeth with no positive history of extraction, and all retained deciduous teeth. Percussion, thermal, and electrical vitality tests were used.

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The assertions and opinions contained in this article are those of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

All carious surfaces found by the visual examination were charted on the standard examination form used in the Navy. Filled surfaces and missing teeth were also charted. No attempt was made to classify degree of dental caries. If the surface offered no resistance to the explorer tip, it was counted as a carious surface. Faulty restorations were also counted as carious surfaces. Fractured surfaces not involved with caries were not considered as surfaces to be restored.

Anterior teeth were considered as having four surfaces and posterior teeth five surfaces in determining the decayed, missing and filled, tooth surface numbers. Missing teeth were given a value of four[1] in the DMF calculation. Because of the age variations throughout the sample no third molar experience was utilized in the data for this report. Such information was gathered during the examination but was later discarded when no satisfactory value could be assigned in the DMF calculation. The inability to establish a DMF value utilizing the third molars was due to a lack of knowledge as to whether they were removed because of caries, nonfunction, or impaction and because many third molars were unerupted in the younger men.

All the radiographs were diagnosed and carious surfaces not discovered during the visual examination were charted on the examination form. A surface was deemed carious if it showed evidence of decalcification up to the dentino-enamel junction. Enamel lesions not large enough to restore were not counted.

Each individual was then asked to complete the following questionnaire: (1) name and Navy identification matter, (2) age at nearest birthday, (3) regular or reserve Naval status, (4) if regular, date first joined; if reserve, date called to active duty, (5) amount of continuous active duty to date, (6) whether or not a recruit training center was attended during the current tour of duty; if so which one, (7) the state resided in longest before becoming 21 years of age.

The bottom of each questionnaire was used as a tabulation sheet for additional information which was collected from all other dental records. From the information on the questionnaire the men were grouped into the following four areas with time and status in the Navy the governing factors:

Group I: Men in the regular Navy with 1 to 12 months service.

Group II: Men in the regular Navy with 13 to 48 months continuous service.

Group III: Men in the regular Navy with 49 or more months of continuous service.

Group IV: Men called to active duty from reserve sources from July 1, 1950 through July 1, 1951.

Men not satisfying these basic time units were rejected at this level.

A total of 1570 officers and enlisted men were examined for this study. The examinations were carried out by one person, and the radiographs were exposed and diagnosed by the same individual.

DISCUSSION

In general, the forecast of the needs (Table I) was projected by analyzing the existing conditions in each group. However, the sum of the forecast needs does not represent the total needs of the Naval Service. This is obvious because any change which satisfies the total need in Group I during recruit training, will reduce the needs of Groups II and III to that of new carious surfaces and new supporting tissue pathology. There is some correlation between new carious surfaces and advancing age regardless of the dental care factor.

To determine the representative value of the sample as related to the Naval Service at large, a study was made in which the population of the various states was compared to the population of the United States on a percentage basis. The percentages per state of the total sample were then compared with the above study. Since no significant difference between these percentages was noted, it was concluded that the sample is represenative of the Naval Service at large.

The forecast for Group I represents the actual needs of recruits entering the regular Naval Service. In addition to their needs found upon examination, the forecast also includes all prior Naval treatment. The majority of Group I came to the ship directly from two Naval Training Centers. From the study of the existing carious surfaces it was determined that these two centers completed approximately 10 per cent and 20 per cent respectively of the recruits' dental needs.

The forecasts for Groups II, III, and IV were based on actual needs as shown by examination. Since Group IV consisted of men called to active duty from civilian occupations on or after July 1, 1950, this group had received little or no Naval dental care prior to reporting aboard for duty.

The forecast for all appliance needs was based upon the oral examinations. In the case of Group I, the forecast also includes all work previously accomplished by Naval dentists. Only essential cases were considered, such as those with insufficient teeth to properly masticate their food and those needing an appliance essential to their duties. Individuals not having at least four molar teeth (one maxillary and one mandibular on both right and left sides) in functional occlusion were considered as having insufficient teeth to properly masticate their food. It is possible that in some cases the forecast needs for removable partial dentures could be filled by fixed partial dentures. The number of repairs and reconstructions needed in the existing appliances was based on the total number of people in the sample rather than on the particular group studied.

During the examinations the oral hygiene was considered and a type assigned to each case, as follows:

Type 1: These mouths showed a good quality of oral care. The tooth surfaces were free from food film, the gingival crevices and interproximals were free of debris and displayed no signs of food irritation. The soft tissue was firm and of good color. No acute or chronic infection was present.

Type 2: These mouths could be restored to health by the proper oral hygiene measures initiated by the individual. These mouths had food film on the teeth. Soft tissues were irritated by food debris. Mild gingivitis was apparent showing a lack of care on the individual's part.

Type 3: These mouths required the intervention of a dentist to restore them to health because of an acute soft tissue condition or a condition of extreme bone loss.

Oral hygiene types were forecast in terms of the number in need of oral hygiene instruction. This emphasizes the approach necessary to reduce the number of Type 2 mouths. About 60 Type 3 mouths, which demanded treatment upon sight, were found in the total sample.

The men primarily in need of instruction are those having Type 2 mouths, since it is this type mouth which can become Type 1 or Type 3 simply by an individual's own effort or lack of effort.

TABLE I
TYPE AND FREQUENCY OF DENTAL NEEDS

	GROUP I	GROUP II	GROUP III	GROUP IV
Number of surfaces to be restored per 1000 men*	9750 ±550	6050 ±780	4180 ±400	4550 ±480
Number of teeth now missing per 1000 men*	2290 ±110	2430 ±230	3280 ±310	3490 ±350
Number of full dentures needed per 1000 men†	13 ± 3	32 ± 5	16 ± 4	36 ± 6
Number of removable partial dentures needed per 1000†	67 ± 8	43 ± 6	40 ± 6	70 ± 8
Number of repairs and reconstructions per 1000†	10 ± 3			
Number needing oral hygiene instruction per 1000 men†	260 ±14	260 ±14	150 ±11	210 ±13

*1000 M and ± = 1000 σ M

†1000 f and ± = 1000 σ f. $\quad f = N\sqrt{\frac{pq}{N}}$

Table II gives the filled surfaces, DMF and the per cent of the group now possessing appliances. By analyzing the trends of the means through Groups

TABLE II
TREATMENT TRENDS

	GROUP I	GROUP II	GROUP III	GROUP IV
N	748	276	256	275
Mean filled surfaces*	10.11 ±0.70	10.10 ±1.15	15.84 ±1.41	14.82 ±1.41
Mean DMF*	21.40 ±1.25	21.30 ±2.25	29.30 ±2.94	29.45 ±2.84
Appliances now in place per 1000 men				
Fixed partial dentures†	31 ± 5	80 ± 9	105 ± 10	76 ± 8
Removable partial dentures†	34 ± 5	47 ± 7	74 ± 8	76 ± 8
Full dentures†	15 ± 4	29 ± 5	55 ± 7	61 ± 8

*Values for the Confidence Limits at the 5 per cent level. C. L. 5 per cent = 1.96 σ M

†See Table I.

TABLE III

CRITICAL RATIO TEST OF THE MEAN DIFFERENCES BETWEEN 50 PAIRS FROM GROUPS III AND IV MATCHED FOR AGE

FUNCTION	GROUP WITH HIGHER INCIDENCE	CRITICAL RATIO
Filled surfaces	III	0.18
Missing teeth	IV	1.18
DMF	IV	1.38
Carious surfaces	IV	2.67

$$M_d = \frac{\Sigma d}{N} \qquad \sigma M_d = \sqrt{\frac{\Sigma d^2}{N(N-1)}} \qquad C.R. = \frac{M_d}{\sigma M_d}$$

I, II, and III, it is apparent that after the initial dental care received during recruit training, the men received very little additional care until they passed into their second or later enlistments.

After regular Navy personnel enter Group III they are soon in an equal or a better position than their reserve shipmates, as shown by Table III. Table III is compiled from matched pairs from Groups III and IV.

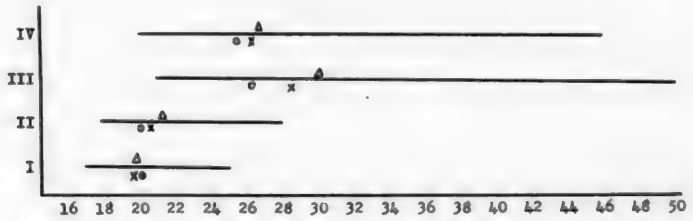

Fig. 1.—Graph showing mean (△), median (×), mode (○), and range for the four groups. The mean of each group is statistically independent of the others at better than the 1 per cent level.

An inspection of Table IV re-emphasizes the importance of dental care as administered by a dentist. The correlations between advancing age and DMF, age and missing teeth, and age and unfilled carious surfaces are very small. On the other hand, there is some correlation which is not measured in a direct manner, between advancing age and the caries process. It is reflected, however, in the greater correlation between age and DMF in Groups III and IV. Fig. 1, showing age range by groups, indicates that Groups III and IV have the largest

TABLE IV

THE CORRELATION BETWEEN AGE AND TOTAL DMF; AGE AND MISSING TEETH; AND AGE AND CARIOUS SURFACES

		GROUP II	GROUP III	GROUP IV
N		276	256	275
Total DMF/age	r =	.12	.19	.28
Missing teeth/age	r =	.02	.17	.17
Carious surfaces/age	r =	—	—	.03

All correlations are positive.

$$r = \frac{N\Sigma fd_x d_y - \Sigma d_x \Sigma d_y}{\sqrt{N\Sigma fd_x^2 - \Sigma(fd_x)^2}\ \sqrt{N\Sigma fd_y^2 - \Sigma(fd_y)^2}}$$

age variations. This accounts for the larger correlation. No correlation was found between age and carious surfaces as observed during examination because of the dental care factor. The number of true carious surfaces is also represented by filled surfaces and missing teeth.

SUMMARY AND CONCLUSIONS

1. The utilization of data derived from the examination of 1570 Navy men has been analyzed by statistical methods and used as a basis for predictions of treatment needs as shown in Table I.

2. By the use of Table I, and with a knowledge of a groups naval status, the amount of treatment needed for any number of men may be calculated.

3. This study should be of value in the allocation of dental facilities in order to derive the greatest possible good from available dental personnel.

4. The dental condition of regular Navy personnel with over four years of service is, in general, better than that of the comparable reserve personnel who have been under other than naval care.

5. The factor of dental care as applied by individual dentists is the best means of controlling the over-all dental conditions. The individual's own personal care is the next most important factor. Age, as such, influences the over-all dental condition as found by less than 10 per cent.

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THE EVANSTON DENTAL CARIES STUDY

IX. The Value of Roentgenological vs. Clinical Procedures for the Recognition of Early Carious Lesions on Proximal Surfaces of Teeth

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IT IS a well-known fact that it is impossible to detect all proximal surface lesions by a clinical examination. It is equally well recognized that the x-ray is a valuable aid for the visualization of early enamel lesions involving the proximal surfaces. The purpose of this study is to determine the frequency in which carious lesions on the proximal surfaces may be demonstrated in the x-ray film and not detected during clinical examination. Data for this analysis have been completed from the records of 12,014 Evanston and Oak Park children of ages 6 to 8 and 12 to 14 years.

The literature contains reports of several previous studies. Fixott[1] estimated that 25 per cent to 40 per cent more carious lesions can be demonstrated to exist in the children's mouths by the use of the radiographic method than were observed by the clinical examinations. Smith[2] in comparing the results of the clinical and x-ray examinations of 210 children found that the x-ray revealed additional lesions in 67.7 per cent of the children. Barr[3] compared the number of proximal lesions observed clinically with those demonstrated by means of roentgenograms on 157 children. The total number of proximal cavities found by both methods was 1,117. The x-ray films revealed 47.2 per cent lesions which were not found in the clinical examination. While examining an unstated number of students Delabarre[4] found 1,842 carious lesions by clinical procedure and an additional 1,960 lesions by x-ray. Trithart and Donnelly[5] studied clinically, and with bitewing films, 449 children in three different schools, however, they retained only 254 children after eliminating those in which all proximal surfaces could not be clearly visualized in the films. In the three schools respectively they found 54.5 per cent, 57.6 per cent and 111 per cent more lesions by the use of x-ray. They concluded that an examination to determine the presence of dental decay is not complete unless x-ray films are included. Chilton and Greenwald[6] examined 2,540 children between the ages of 5 to 18 years, but they selected for radiographic survery about every third child bringing their total number of children so examined to 729. They found 2,190 carious lesions by clinical methods and 2,762 by roentgenologic procedures. They summarized "that when studies are undertaken to determine the efficacy of measures taken to prevent or retard caries, the factor of chance comes into play, and the statistical significance of oftentimes small differences in the average number of carious teeth per child may readily be influenced by more accurate diagnosis

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of carious lesions. For that reason, x-ray examinations assume practical importance in public health surveys conducted for dental research purposes." In addition, they concluded "that dental public health surveys to determine the dental needs of school populations or surveys to compare group dental status, do not require roentgenographic examinations, but that dental surveys, conducted for research purposes, require roentgenographic examinations for proper interpretation of results."

METHOD

In the Evanston Dental Caries Study, during the years 1946 to 1950 inclusive 15,209 children in the age groups of 6, 7, 8, 12, 13, and 14 from 24 schools in the study area and 12 schools in the control community were examined for dental caries by detailed clinical and x-ray methods. The clinical examinations were conducted by well trained dentists with the use of sharp explorers, mouth mirrors, and adequate light after the teeth had been freed from saliva by the use of the air syringe.

An experienced technician radiographed the entire mouth using 3 films for the maxillary anterior teeth, 3 films for the mandibular anterior teeth, one bitewing film on each side for the 6, 7, and 8 year old children and 2 bitewing films on the older group. The Raper[7] method of adjusting a film which consists of fastening a bitewing tab onto the film packet by means of radioparent scotch tape was adopted for taking the posterior bitewing films.

In order to get better detail in the films and to eliminate as much error as possible the Fitzgerald[8] method is followed which advocates, "The diffusion of detail is minimized by increasing the cone distance; anatomic accuracy is attained by a difference in film placement and angulation and by a slight change in object/film distance." Since the study began we have been using a 16 inch target film distance whereby the film packet is held away from the crowns of the teeth by the use of cotton rolls so that the plane of the film parallels the long axes of the teeth. The surfaces of the film are never bent to follow the contour of the alveolar ridge. In order to secure the maximum detail in each film a uniform exposure time is observed for each area and processed at a constant temperature (68° F.) and time (5 minutes).

The findings are always rechecked by a co-worker to insure proper interpretation and tabulation.

FINDINGS

The 6, 7, and 8-year-old group exhibited a total of 2,101 carious lesions on the proximal surfaces of the permanent teeth. By clinical examination 647 or 30.79 per cent cavities were detected while 1,454 or 69.21 per cent additional lesions were revealed by the x-ray films. It is interesting to note that when these age groups are considered separately, the 6 year olds have the highest percentage (83.90) disclosed by x-ray findings only. In the 7-year-old group 79.04 per cent of proximal lesions were demonstrated by x-ray findings only and in the 8 year 58.78 per cent were discovered in the films. (Table I.)

TABLE I

PROXIMAL SURFACES OF PERMANENT TEETH OF 12,014 CHILDREN HAVING CARIES RECOGNIZED BY (A) CLINICAL AND (B) X-RAY ONLY

AGE	LESIONS RECOGNIZED CLINICAL EXAM		LESIONS RECOGNIZED X-RAY ONLY		TOTAL LESIONS RECOGNIZED	
	NO.	PER CENT OF TOTAL	NO.	PER CENT OF TOTAL	NO.	PER CENT
6	19	16.10	99	83.90	118	100
7	196	20.96	739	79.04	935	100
8	432	41.22	616	58.78	1,048	100
Total 6, 7, 8	647	30.79	1,454	69.21	2,101	100
12	2,501	44.45	3,126	55.55	5,627	100
13	4,349	38.38	6,983	61.62	11,332	100
14	4,165	34.78	7,810	65.22	11,975	100
Total 12, 13, 14	11,015	38.07	17,919	61.93	28,934	100
Grand total for all ages	11,662	37.58	19,373	62.42	31,035	100

The 12, 13, and 14-year-old group had a total of 28,934 carious lesions in the proximal surfaces of the permanent teeth. By clinical examination 11,015 cavities were found while the x-ray survey revealed additional 17,919 or 61.93 per cent of the total. When this group is separated according to age we find that in the 12-year-old children 55.55 per cent of the lesions were not found in the clinical study. In the 13 and 14-year-old groups we visualized 61.62 per cent and 65.22 per cent respectively in the films which were not observed clinically. (Table I.)

For the deciduous dentition the comparison between the radiographic and clinical findings is made on data compiled from the records of 9,293 children. The younger age group composed of 6, 7, and 8 year olds had a total of 32,718 carious lesions on the proximal surfaces of the deciduous teeth. Of this number 15,096 or 46.14 per cent were revealed by the roentgen study in addition to those observed during clinical examination. The older group of children, i.e., the 12, 13, and 14 year olds, exhibited a total of 1,023 proximal surface lesions. Here again, the laboratory survey revealed 408 or 39.88 per cent of the total number of carious areas.

TABLE II

PROXIMAL SURFACES OF DECIDUOUS TEETH OF 9,293 CHILDREN HAVING CARIES RECOGNIZED BY (A) CLINICAL AND (B) X-RAY ONLY

AGE	LESIONS RECOGNIZED BY CLINICAL EXAM		LESIONS RECOGNIZED BY X-RAY ONLY		TOTAL LESIONS RECOGNIZED	
	NO.	PER CENT OF TOTAL	NO.	PER CENT OF TOTAL	NO.	PER CENT
6	5,340	54.93	4,381	45.07	9,721	100
7	7,214	55.34	5,821	44.66	13,035	100
8	5,068	50.87	4,894	49.13	9,962	100
Total 6, 7, 8	17,622	53.86	15,096	46.14	32,718	100
12	375	60.00	250	40.00	625	100
13	176	58.47	125	41.53	301	100
14	64	65.98	33	34.02	97	100
Total 12, 13, 14	615	60.12	408	39.88	1,023	100
Grand total for all ages	18,237	54.05	15,504	45.95	33,741	100

When these two groups were examined according to their respective ages we found that the roentgenologic survey revealed 45.07 per cent additional lesions in the 6 year olds, 44.66 per cent in the 7, 49.13 per cent in the 8, 40.00 per cent in the 12, 41.53 per cent in the 13, and 34.02 per cent in the 14-year-old children. (Table II.)

DISCUSSION

It should be clearly stated that these data have no bearing upon the value of the addition of fluorides to the Evanston water. This particular study is directed solely to the evaluation of roentgenologic procedures in the determination of the prevalence of dental caries. When the comparison between clinical and roentgenologic findings is made on such a large number of children, by the same group of examiners under what is thought to be very favorable conditions, we begin to approach what may be considered an average ratio for any one individual patient of comparable age.

TABLE III

SUMMARY OF LESIONS ON PROXIMAL SURFACES OF DECIDUOUS AND PERMANENT TEETH RECOGNIZED BY (A) CLINICAL AND (B) X-RAY ONLY

	LESIONS RECOGNIZED BY CLINICAL EXAM		LESIONS RECOGNIZED BY X-RAY ONLY		TOTAL LESIONS RECOGNIZED	
	NO.	PER CENT OF TOTAL	NO.	PER CENT OF TOTAL	NO.	PER CENT
Deciduous teeth	18,237	54.05	15,504	45.95	33,741	100
Permanent teeth	11,662	37.58	19,373	62.42	31,035	100
Grand total	29,899	46.16	34,877	53.84	64,776	100

We believe it to be quite obvious why the 6-year-old children had the largest percentage of lesions in permanent teeth demonstrated in the x-ray findings with succeeding lesser amounts in both the 7 and 8 year olds. A corresponding range is found to exist in the 12, 13, and 14 year group with 55.55, 61.62 and 65.22 per cent respectively. The time of eruption of the various units of the permanent dentition in relation to the age group examined is such that normally we should expect to find only very early carious lesions in the enamel of proximal surfaces of these particular ages showing the highest percentages. When the exposure time of tooth surfaces to an environment compatible with caries activity is increased it is logical to expect such lesions to be larger and much more readily observed by clinical methods.

When the data for both deciduous and permanent dentitions for 12,014 children are summarized and we find that the x-ray disclosed 53.84 per cent of the total number of carious lesions observed by both clinical and x-ray methods, we at once envision the importance of the laboratory technique for the appraisal of caries prevalence (Table III). For the school survey programs which are primarily directed toward encouraging the parents to seek dental treatment for their children x-ray studies are not necessary. However, it appears that an oral examination cannot be complete without a thorough clinical and laboratory set of procedures. We believe it extremely important to employ both clinical and x-ray techniques in any study program which is directed toward the determination of the prevalence or the control and reduction in the rate of caries attack.

SUMMARY

Data collected from 12,014 children have been presented and analyzed for the value of roentgenologic procedures as a means of visualization of carious lesions on the proximal surfaces of both deciduous and permanent dentitions. A total of 64,776 carious lesions were recorded. Of this number, 34,877 separate carious areas (53.84 per cent) were detected by roentgen studies which were completely overlooked in what is considered to be a thorough clinical examination.

The authors gratefully acknowledge the valuable assistance given by all members of the Evanston Dental Caries Study team in the collection of the data.

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THE EVANSTON DENTAL CARIES STUDY

X. The Caries Experience Rates of 6, 7, and 8-Year-Old Children With Progressively Increasing Periods of Exposure to Artificially Fluoridated Water

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IN A recent report of this study a reduction in the dental caries experience rates of the permanent teeth of a group of 6 to 8-year-old children was reported following exposure to a fluoridated water for a period of 12 to 22 months.[1] Also it was reported that the dental caries experience rates of the deciduous teeth of this group was not lessened following 12 to 22 months exposure to the fluoridated water.[1] This lack of a reduction was thought to be due to the fact that these deciduous teeth were fully erupted and in the mouth prior to the addition of sodium fluoride to the water. Therefore, it now seems logical to determine whether there might be a significant difference in the caries rates among children whose deciduous teeth were either in early stages of eruption or had recently erupted at the time fluorine was placed in the water as compared with children whose deciduous teeth were erupted and in the mouth for various lengths of time when the communal water was fluoridated.

As sodium fluoride was added to the Evanston water in February of 1947 and groups of 6 to 8-year-old children were examined in 1946, 1948, 1950, and 1951 a favorable opportunity was afforded for comparing the dental caries experience rates of children exposed to fluoridated water for various lengths of time.

METHODS

This report compares the caries experience rates of the deciduous and permanent teeth of four groups of 6, 7, and 8-year-old Evanston children. The first, or base line group (1946), had no exposure to fluoridated water. The second (1948), third (1950), and fourth (1951) groups were exposed to the fluoridated water from 12 to 22, 35 to 46, and 47 to 58 months, respectively, at the time of examination. It should be noted, as shown in Table I, that some of the 1951 group of children were 9 months of age at the time sodium fluoride was placed in the water. Enamel formation was taking place at this time on the maxillary and mandibular permanent incisors, canines, and first molars, however, the calcification of the crowns of the deciduous teeth had been completed. In the younger children only the deciduous incisors had begun the process of eruption, but most of the deciduous teeth were exposed to tissue fluids containing fluorides for several months before erupting into the mouth.

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TABLE I

PERIOD OF EXPOSURE TO ARTIFICIALLY FLUORIDATED WATER AND AGE RANGE OF EACH GROUP OF CHILDREN AT THE TIME OF INITIAL FLUORIDATION

	YEAR	NO. OF EXAMS.	F.	MONTHS OF F. WATER	AGE RANGE IN MONTHS AT TIME OF FLUORIDATION
(Base Line Group)	1946	1,991	0.0	none	---------
	1948	2,034	1.0	12-22 mos.	45-90 mos.
	1950	1,244	1.0	35-46 mos.	21-67 mos.
	1951	1,853	1.0	47-58 mos.	9-55 mos.

A thorough clinical examination supported by 6 anterior roentgenograms and 2 posterior bitewing films was made on all children. All examinations were performed in the schools with adequate dental equipment and proper lighting. The schedule was arranged to allow ample time for a thorough examination. The recognition of carious lesions, fillings, and conditions of investing tissues, was accomplished by the same dentists for the four groups. The opinion of at least two dentists was obtained before the final analysis of the case was made. Diagnosis of an immune case was made only on the basis of complete x-ray films. There were no carious lesions or fillings in the deciduous teeth of immune cases and all or a majority of deciduous teeth were present in the mouth. A case of mixed dentition, with no caries experience, was considered a questionable immune case rather than one of complete immunity. There were no questionable cases included in this or in previous reports. All cases with orthodontic appliances were eliminated from the immune case tabulation though no evidence of caries experience was observed.

The clinical and roentgenologic data were transferred to code sheets so that findings concerning deciduous and permanent teeth, teeth lost physiologically, and those teeth lost due to caries were properly recorded. This coded material was segregated and classified mechanically at the Bureau of Vital Statistics, Dept. of Health, Springfield, Ill.

The data analyzed for this report of the groups considered are limited to the following: (1) the decayed, extracted and filled rate of the deciduous teeth, (2) the number of children with deciduous dentitions immune to caries at the time of examination, (3) the decayed, missing and filled rates of the permanent teeth, (4) the caries experience of the occlusal surface of the first permanent molar teeth, (5) the caries experience of the maxillary and mandibular anterior permanent teeth, (6) the precarious lesions found on surfaces of all permanent teeth.

FINDINGS

Deciduous Teeth.—The def rate of the 1951 group of 6 year olds is not significantly less per 100 children than that of the 1946 base line group. The 1951 group of 6 year olds were in an age range of 9 to 31 months at the time sodium fluoride was placed in the water. It is logical to assume that if a caries reduction is found it would appear in this group. There was a slight increase in the caries rate of the deciduous teeth of the 7 year old over the base line group which is not considered significant. In contrast our findings for this same age group after 12 to 22 months of water fluoridation in 1948 revealed a

TABLE II

DECAYED AND EXTRACTED (DUE TO CARIES) AND FILLED DECIDUOUS TEETH RATES ARE PER HUNDRED CHILDREN

YEAR	NO. EXAMS.	TIME OF EXPOSURE TO 1 PPM OF F.	AGES 6	7	8	(6-8)
1946	1,991	None	482.86	550.06	577.17	536.70*
1948	2,034	12-22 mos.	507.80	541.05	620.22	556.35*
Age range at time of initial fluoridation			(45-66 mos.)	(57-78 mos.)	(69-90 mos.)	
1950	1,244	35-46 mos.	478.37	668.04	653.51	599.89
Age range at time of initial fluoridation			(21-43 mos.)	(33-55 mos.)	(45-67 mos.)	
1951	1,853	47-58 mos.	449.63	558.26	674.57	560.82
Age range at time of initial fluoridation			(9-31 mos.)	(21-43 mos.)	(33-55 mos.)	

Differences are not statistically significant.

*It should be noted that the caries rates per 100 children for the 6-8 year olds as a group shown in this report, vary slightly from those shown in previous reports. The new averages were obtained by taking a simple arithmetical mean of the individual caries rates of the 6-, 7- and 8-year-old children. The group averages, shown in previous reports, represents weighted averages of the individual mean caries rates. Inasmuch as the composition of the groups of children with respect to the number of 6-, 7- and 8-year-olds varies from year to year, it was felt that unweighted group averages form a more sound basis for comparison of group caries rates between years.

reduction of approximately the same amount. In 1950 (35 to 46 months exposure) the rate increased to 668.04 but dropped to 558.26 in 1951. The 1951 rate is less than the 1950 rate but higher than the 1946 and 1948 rates. It is of interest to note that the rates for the 1951 6 and 7-year-old children are almost the same as the 1946 rate for these two ages. The 8-year-old children demonstrated a percentage increase in def rates for the deciduous teeth of 7.46 in 1948, 13.23 in 1950, and 16.87 in 1951, when compared to the base line. Table II.

Immune Deciduous Dentitions.—Although the number of caries immune deciduous dentitions per 100 children fluctuated for the four groups the 1951 group rate was less for all three ages than the base line group. Specifically the rates of the 6 year olds dropped from 21.04 in 1946 to 15.87 in 1948. The rate dropped further in 1950 to 13.17 but rose to 16.79 in 1951. The 7 year olds indicated a decrease from 11.33 in 1946 to 8.71 in 1948 to 3.87 in 1950 then rose to

TABLE III

IMMUNE DECIDUOUS DENTITIONS AT THE TIME OF DENTAL EXAMINATION RATES ARE PER HUNDRED CHILDREN

YEAR	NO. EXAMS.	TIME OF EXPOSURE TO 1 PPM OF F.	AGES 6	7	8	(6-8)
1946	1,991	None (base line)	21.04	11.33	6.09	12.82*
1948	2,034	12-22 mos.	15.87	8.71	8.18	10.92*
Age range at time of initial fluoridation			(45-66 mos.)	(57-78 mos.)	(69-90 mos.)	
1950	1,244	35-46 mos.	13.17	3.87	4.30	7.11
Age range at time of initial fluoridation			(21-43 mos.)	(33-55 mos.)	(45-67 mos.)	
1951	1,853	47-58 mos.	16.79	10.66	5.74	11.06
Age range at time of initial fluoridation			(9-31 mos.)	(21-43 mos.)	(33-55 mos.)	

Differences are not statistically significant.

*See footnote, Table II.

10.66 in 1951. The 8-year-old children indicated a rise from the 1946 rate of 6.09 to 8.18 in 1948 then dropping to 4.30 in 1950. In 1951 the rate rose to 5.74 cases per 100 children. Table III. It would be reasonable to assume that when the caries attack rate was raised or lowered a change would likewise be observed in the immune deciduous dentition cases per hundred children. This was mainly true for the 6 and 7 year olds (exceptions 6 year olds 1950 and 7 year olds 1948) while the 8-year-old children followed the prediction only in the 1950 group (Table II & III).

We believe these slight variations can be expected to occur from one year to the next and probably are without significance.

Permanent Teeth—Decayed, Missing, and Filled.—The rates for the permanent teeth of the 1951 group indicated a percentage reduction in dental caries experience of 73.61 for the 6 year olds; 56.37 for the 7 year olds, and 35.42 for the 8 year olds when compared to the original base line figures. The three age groups demonstrated a constant drop in dental caries experience except the 1951 8 year group which showed a very slight increase when compared to the 1950 8-year-old group. It is interesting to note that the 1950 rate of the 8-year-old children is almost exactly the same as the rate of the 7-year-old children of the base line group in 1946 (Table IV).

TABLE IV
DECAYED AND MISSING (EXTRACTED DUE TO CARIES) AND FILLED PERMANENT TEETH
RATES ARE PER HUNDRED CHILDREN

YEAR	NO. EXAMS.	TIME OF EXPOSURE TO 1 PPM OF F.	AGES 6	7	8	(6-8)
1946	1,991	None (base line)	46.85	153.49	249.93	149.76*
1948	2,034	12-22 mos.	23.54	103.58	194.09	92.07*
Age range at time of initial fluoridation			(45-66 mos.)	(57-78 mos.)	(69-90 mos.)	
1950	1,244	35-46 mos.	15.33	80.93	153.52	83.26
Age range at time of initial fluoridation			(21-43 mos.)	(33-55 mos.)	(45-67 mos.)	
1951	1,853	47-58 mos.	12.36	66.97	161.40	80.24
Age range at time of initial fluoridation			(9-31 mos.)	(21-43 mos.)	(33-55 mos.)	

Differences are not statistically significant.
*See footnote, page 348.

First Permanent Molar Occlusal Surface Caries Experience.—

Occlusal surfaces with pit and fissure caries or fillings: It is apparent that a reduction of 73.61 per cent in the dental caries experience rate of the permanent teeth of a group of 6-year-old children after exposure to fluoridated water from 47 to 58 months would be primarily a reduction of dental caries of the first permanent molar teeth. The reduction in dental caries experience limited to the occlusal surface of the first permanent molar teeth of the 6 year olds was 80.21 per cent when comparing the 1951 group to the 1946 group. The 7 year olds demonstrated a 66.97 per cent reduction and the 8 year olds, 44.92 per cent. The rate of the 8 year olds in the 1951 group was slightly higher than the 1950 rate but was less than the 1946 and 1948 rates. Again, this may be considered

TABLE V
FIRST PERMANENT MOLAR TEETH WITH OCCLUSAL SURFACE PIT AND FISSURE CARIES OR FILLINGS
RATES ARE PER HUNDRED CHILDREN

YEAR	NO. EXAMS.	TIME OF EXPOSURE TO 1 PPM OF F.	AGES 6	7	8	(6-8)
1946	1,991	None (base line)	40.13	130.03	206.35	125.50*
1948	2,034	12-22 mos.	19.97	77.32	145.00	80.76*
Age range at time of initial fluoridation			(45-66 mos.)	(57-78 mos.)	(69-90 mos.)	
1950	1,244	35-46 mos.	10.33	52.32	108.59	57.08
Age range at time of initial fluoridation			(21-43 mos.)	(33-55 mos.)	(45-67 mos.)	
1951	1,853	47 58 mos.	7.93	42.94	113.64	54.84
Age range at time of initial fluoridation			(9-31 mos.)	(21-43 mos.)	(33-55 mos.)	

Differences are not statistically significant.
*See footnote, page 348.

as a normal fluctuation. Here the 7 year olds of 1951 and the 1946 6 year olds have almost the same rates. Also the rate of the 1951 group of 8 year olds and the 1946 7 year olds are somewhat similar (Table V).

Occlusal surfaces free from pit and fissure caries or fillings: It would seem probable that in the same aged group if the occlusal surface caries experience was decreasing the number of occlusal surfaces free from caries experience would be increasing. This was true for the 7 and 8 year olds but not for the 6 year olds. The 6 year olds, showed a reduction in rates for both the carious and free from carious occlusal surfaces (Tables V and VI).

The apparent discrepancy may be accounted for when you recall that we classify a 6 year old as having an age range of from 67 to 78 months, obviously the youngest children have not yet erupted all of the permanent molars. Therefore these teeth do not appear on the chart as being present in the mouth and

TABLE VI
FIRST PERMANENT MOLARS FREE FROM OCCLUSAL SURFACE PIT AND FISSURE CARIES OR FILLINGS
RATES ARE PER HUNDRED CHILDREN

YEAR	NO. EXAMS.	TIME OF EXPOSURE TO 1 PPM OF F.	AGES 6	7	8	(6-8)
1946	1,991	None (base line)	148.37	200.26	180.28	176.30*
1948	2,034	12-22 mos.	136.37	259.18	242.05	212.53*
Age range at time of initial fluoridation			(45-66 mos.)	(57-78 mos.)	(69-90 mos.)	
1950	1,244	35-46 mos.	130.00	268.81	275.00	224.60
Age range at time of initial fluoridation			(21-43 mos.)	(33-55 mos.)	(45-67 mos.)	
1951	1,853	47-58 mos.	124.54	272.97	272.09	223.20
Age range at time of initial fluoridation			(9-31 mos.)	(21-43 mos.)	(33-55 mos.)	

Differences are not statistically significant.
*See footnote, page 348.

TABLE VII
DECAYED AND FILLED MAXILLARY ANTERIOR PERMANENT TEETH
RATES ARE PER HUNDRED CHILDREN

YEAR	NO. EXAMS.	TIME OF EXPOSURE TO 1 PPM OF F.	AGES 6	7	8	(6-8)
1946	1,991	None (base line)	0.65	3.03	8.69	4.12*
1948	2,034	12-22 mos.	0.00	1.67	5.00	2.22*
Age range at time of initial fluoridation			(45-66 mos.)	(57-78 mos.)	(69-90 mos.)	
1950	1,244	35-46 mos.	0.17	0.26	1.56	0.66
Age range at time of initial fluoridation			(21-43 mos.)	(33-55 mos.)	(45-67 mos.)	
1951	1,853	47-58 mos.	0.00	0.75	2.33	1.03
Age range at time of initial fluoridation			(9-31 mos.)	(21-43 mos.)	(33-55 mos.)	

Differences are not statistically significant.
*See footnote, page 348.

cannot be listed as being caries free. Tabulations based on the rate per 100 molar teeth and not as 100 children will undoubtedly show an increase in the number of caries free occlusal surfaces of first permanent molars.

Maxillary and Mandibular Anterior Teeth (canines, first, and second incisors).—The 6 year old rating could be eliminated as there are so few permanent first incisor teeth. The rates for the maxillary anterior teeth for the 7 and 8 year olds in 1951, though much lower than the base line group, increased over the 1950 group. The 1951 8 year olds indicated an increase in the rate compared to the 1950 group. However it was lower than the rate of the 1946 7 year olds. Table VII. The rates for the mandibular anterior teeth fluctuated from 1946 through 1951. However all of the rates are less than 1 DMF tooth per hundred children in the 6 year olds. The 7 year olds ranged from 3.82 in 1946 to 1.37 in 1948 to 2.32 in 1950 and back to 1.05 in 1951. The 8 year olds ranged from 5.19 in 1946 to 2.72 in 1948 to 3.13 in 1950 ending at 3.26 in 1951 (Table VIII).

Permanent Tooth Surfaces With Pre-carious Lesions.—In some cases it is impossible to be absolutely certain whether or not a deep occlusal fissure or

TABLE VIII
DECAYED AND FILLED MANDIBULAR ANTERIOR PERMANENT TEETH
RATES ARE PER HUNDRED CHILDREN

YEAR	NO. EXAMS.	TIME OF EXPOSURE TO 1 PPM OF F.	AGES 6	7	8	(6-8)
1946	1,991	None (base line)	0.86	3.82	5.19	3.29*
1948	2,034	12-22 mos.	0.13	1.37	2.72	1.41*
Age range at time of initial fluoridation			(45-66 mos.)	(57-78 mos.)	(69-90 mos.)	
1950	1,244	35-46 mos.	0.33	2.32	3.13	1.93
Age range at time of initial fluoridation			(21-43 mos.)	(33-55 mos.)	(45-67 mos.)	
1951	1,853	47-58 mos.	0.00	1.05	3.26	1.44
Age range at time of initial fluoridation			(9-31 mos.)	(21-43 mos.)	(33-55 mos.)	

Differences are not statis.ically significant.
*See footnote, page 348.

TABLE IX

PERMANENT TOOTH SURFACES WITH PRE-CARIOUS LESIONS
RATES ARE PER HUNDRED CHILDREN

YEAR	NO. EXAMS.	TIME OF EXPOSURE TO 1 PPM OF F.	AGES 6	7	8	(6-8)
1946	1,991	None (base line)	50.32	96.83	107.65	84.93*
1948	2,034	12-22 mos.	19.70	63.00	68.63	50.44*
Age range at time of initial fluoridation			(45-66 mos.)	(57-78 mos.)	(69-90 mos.)	
1950	1,244	35-46 mos.	12.33	41.75	36.72	30.27
Age range at time of initial fluoridation			(21-43 mos.)	(33-55 mos.)	(45-67 mos.)	
1951	1,853	47-58 mos.	10.52	28.38	47.91	28.94
Age range at time of initial fluoridation			(9-31 mos.)	(21-43 mos.)	(33-55 mos.)	

Differences are not statistically significant.
*See footnote, page 348.

anatomic pit is or is not carious. We have classified these areas as pre-carious because we believe they are at least predisposed to caries attack. Our data reveals a consistent drop in the occurrence of such areas. It should be noted that the 8 year old group of 1951 was the 7-year group of 1950 and that the rates are quite nearly the same. However the rate of 47.91 for the 8 year olds in 1951 is almost as high as the rate of the 6 year olds in 1946 which was 50.32 (Table IX).

The percent difference for the six comparisons of dental caries experience for the 6, 7, and 8 year olds in 1946 and 1951 is shown in Table X.

DISCUSSION

The deciduous teeth dental caries experience rates of the 1951 group were not materially raised or lowered when compared to the base line group of 1946. The fluoride ion may possibly provide some protective action for completely calcified enamel of unerupted teeth. During the post formative but pre-eruptive interim the enamel may absorb fluorine from the tissue fluids. This theory has some merit as there was a slight reduction in the dental caries experience of the deciduous teeth of the 6 year olds of the 1951 group over the base line group. Though some of the 6-year-old children were only 9 months of age at the time of fluoridation the enamel of the deciduous teeth had been calcified though only the deciduous first and second incisors were in the process of eruption.

TABLE X

PER CENT REDUCTION (R) OR INCREASE (I) IN CARIES ATTACK RATES OF 6-7-8 YEAR OLD CHILDREN AFTER 47-58 MONTHS OF EXPOSURE TO ARTIFICIALLY FLUORIDATED WATER

def deciduous teeth	I	4.49
Immunities, deciduous teeth	R	13.73
DMF permanent teeth	R	46.42
Occlusal surface of first permanent molar teeth with caries experience	R	56.30
Occlusal surface of first permanent molar teeth free of occlusal surface caries or fillings	I	28.13
Decayed or filled maxillary anterior permanent teeth	R	75.00
Decayed or filled mandibular anterior permanent teeth	R	59.27
Pre-carious lesions on permanent tooth surfaces	R	65.93

The caries experience rates of the permanent teeth was consistently lessened for all three ages of the 1951 group. The reduction in dental caries experience of the permanent teeth of the 6 year olds is primarily one associated with the occlusal surface of the first permanent molars. There was a reduction in the number of occlusal surfaces with pit and fissure caries or fillings. However the rates of the 6-year-old group did not show an increase in the number of occlusal surfaces of the first permanent molar teeth which were free from dental caries experience. This was explained as being due to the fact that some first permanent molars had not yet erupted, therefore could not be noted as present in the mouth and caries free. Had the rate been expressed as per 100 teeth this discrepancy may not have occurred.

The maxillary and mandibular anterior permanent teeth rates indicated a reduction of approximately 75.00 per cent for the upper anterior teeth and 59.27 per cent for the mandibular anterior teeth. This reduction would be associated primarily with the central and lateral incisor teeth of the 7 and 8 year olds. The rates for the 6 year olds in each group is less than 1 DMF tooth per hundred children.

SUMMARY

The dental caries experience rates of four groups of children exposed to an artificially fluoridated water for various lengths of time has been presented. The last group (1951) was exposed to the water for the longest period of time, 47 to 58 months. The age range of that group at the time of fluoridation of the water was from 9 to 55 months. Although all the deciduous teeth were calcified eruption was not entirely completed at that time. The changes in the dental caries rate of the deciduous dentition for the 1951 group were not considered significant when compared to the 1946 or base line group. The permanent teeth dental caries experience rate of the combined 1951 group of 6 to 8 year old children was 46.42 per cent lower when compared to the base line group.

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EPIDEMIOLOGIC STUDIES IN DENTAL CARIES. V. PLACEMENT OF FILLINGS AS A SOURCE OF STATISTICAL ERROR OF ESTIMATE AS TO DENTAL CARIES PROGRESSION RATES

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EARLIER reported studies from Iowa investigations have shown the manner in which estimates of caries scores may be distorted due to various errors of experiment, judgment, or estimate.[1, 2, 3] This report deals with the errors which may be introduced if no attention is paid to whether or not teeth have been filled prior to the study, or are filled during its course. Quantitative estimates of such error are offered relating to one aspect of the problem.

Operative treatment of dental decay will alter the subsequent course of caries in the treated area. Moreover, the presence of fillings lessens the number of tooth areas susceptible to initial caries attack. Although recognizing the benefit to the patient which dental repair will bring, it seems obvious that one may expect errors of estimate as to caries extent and as to rates of advance if subjects with dental fillings are used in experimental studies relating to the control of caries through nonoperative means. This report deals specifically with the error which may arise through the filling of a cavity when caries extent is being measured in terms of the number of affected tooth surfaces. Such error arises because a filling necessarily is larger than the zone of decay it serves to replace, and frequently will extend to a surface previously free from caries or filling.

To gain a quantitative concept of how much statistical error might arise from the placement of fillings, we have reviewed the dental records of 99 children prior to any operative procedure and then again immediately after repair has been completed. The difference in the number of DF (decayed or filled) surfaces in the contrasted records is taken to represent the degree in which the dental fillings had involved surfaces not previously affected by caries.

PRESENTATION OF DATA

The subjects for the study were children coming for care to the pedodontic clinic of the Dental College Infirmary. The records of 150 such children were chosen serially from the files. From these, 99 records were found which offered comparison of the number of carious tooth surfaces before the child had had any dental repair, and the corresponding number immediately after the recognized cavities had been filled. Records were limited to those subjects regarding whom not more than 90 days had elapsed between the

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initial and postoperative examinations. These records have served as the source of data from which our conclusions in this regard have been drawn.*

The numerical data are shown in Table I and can be summarized as follows: Of the 99 children's records, 60 per cent showed an advance in the number of affected (DF) tooth surfaces attributable solely to the placement of fillings. Among those with increased scores, the median increase was 2 surfaces for each mouth; one-fourth of those showing increases had four added surfaces. When the degree of increase was compared with the number of carious surfaces initially present, the average degree of increase was 25 per cent, and one-fourth of the subjects with increase had 50 per cent or more surfaces added to their DF scores because of the placement of fillings.

TABLE I

CHANGES IN THE NUMBER OF CARIOUS OR FILLED TOOTH SURFACES BROUGHT ABOUT SOLELY THROUGH THE PLACEMENT OF DENTAL FILLINGS

	AGGREGATE	PER CENT	RANGE	MEDIAN	MID-50%
Age (years)*			2¼-13	6¼	4½-8
Interval between pre- and postoperative examinations (days)			1-90	36	20-56
Carious surfaces at the first examination					
a) whole group					
total number of DF surfaces	913				
number of DF surfaces in each mouth			1-37	7	4-13
b) among those whose DF scores later were greater after placement of fillings					
total number of DF surfaces	629				
number of DF surfaces for each mouth			1-37	10	5-14
Incidence of increase in DF scores due solely to the placement of fillings					
a) whole group					
number of added DF tooth surfaces	153				
per cent of initial value		16.8			
number of mouths with added DF surfaces	59				
per cent of total group		60			
number of new DF surfaces for each mouth			0-11	1	0-2
per cent increase for individual mouth			0-100	10	0-29
b) those whose DF scores were greater after placement of fillings					
number of added DF tooth surfaces	153				
per cent of initial value		24.3			
number of new DF surfaces for each mouth			1-11	2	1-4
per cent of initial value			3-100	25	13-50

*49 boys and 50 girls used as subjects.

DISCUSSION

The customary practice is to fill dental cavities once they have been discovered. Through such treatment one may expect to preserve the tooth which has been affected by caries. The placement of the filling tends to check the

*These data were assembled by Dr. John J. Cannon and were used by him as a basis for a thesis prepared in partial fulfillment of requirement for his degree of Master of Science.

further development or progression of caries in the area so treated. Likewise, in some areas of hypoplastic enamel, where caries is prone to occur, recommended practice calls for the placement of fillings even though decay cannot yet be diagnosed with certainty (sometimes even though it is assumed that the area still is noncarious) as a measure for the prevention of tooth decay. Although such measures generally are accepted as desirable for the welfare of the patient, the placement of fillings will interfere in various ways with the conduct of a controlled clinical study of the spontaneous pattern of caries evolution, or of the effect of specific agents on the progress of tooth decay. Sources of some of the errors introduced through the placement of fillings are as follows:

1. The placement of a filling lessens the susceptibility of a tooth surface to further decay. If fillings have been placed, the degree of possible response to any agent being tested will be lessened correspondingly for the mouth as a whole, and one can expect data as to caries advance to be modified accordingly.
2. Once a filling has been placed, an observer can no longer judge whether the area of repair had been demonstrably carious, nor can he observe the subsequent rate, regularity or degree of advance of caries in that particular cavity. Advance of caries is as significant a measure of caries attack as is the number of cavities which become diagnosable during a given period of time. If observations are limited to the counting of areas which develop caries during the course of the study without observation of the course of cavities already present, the results will tell only a part of the story of caries progression.
3. If dental lesions are filled during the course of a clinical study, the examiner will not be able to distinguish between the effect which the filling of the cavity may have had on regional and general caries advance and the effect he may wish to attribute to the agent whose caries-potentiating power he is testing.
4. A filling necessarily is larger than the area of carious enamel and dentin it is designed to repair. The extent of this difference on the DF surface count has been demonstrated for one child population in this report.

Because of these and other reasons, studies dealing with rates of caries advance either should be limited to subjects who have had no reparative dental care prior to the study and who receive none through the course of the study, or else the examiner should weight his findings quantitatively so as to make allowance for the effect which reparative measures may have had on the results. To make such weighted observations, studies such as the one reported herewith will be necessary as basis for estimate. For similar reasons, one should not attempt to make quantitative comparisons of caries incidence among different populations, unless the nature and degree of reparative dental therapy for each group has been equated.

To contest the foregoing statements, it might be stated that one can avoid errors due to the size of the fillings if he limits his consideration to the number of teeth which have been affected by caries rather than to the number of affected tooth surfaces. Even though such a generalization may be true, the limitation of observations lessens the sensitivity of the study to such an extent that only gross changes (e.g., the number of teeth not previously affected by caries) can be measured by the examiner. Moreover, it is important to keep in mind that different teeth within the same mouth show wide variability as to their susceptibility to caries attack. To compare the rate of caries attack for anterior teeth from one subject with that for posterior teeth from another mouth is not justifiable as a statistical procedure. Neither is it justifiable to compare rates of caries progression for subjects whose teeth show minimal incidence of caries with others who at the outset of the study have experienced caries already in most of the susceptible teeth in the mouth. For these reasons, it is important in studies of comparative rates of caries progression to include only subjects who initially have similar caries experience in analogous teeth and also similar posteruptive age of the teeth being studied.

It might be stated that one can avoid numerous potential errors of estimate if the examiner limits consideration to whether or not a carious lesion is present on a given tooth surface, without regard for its size or its subsequent course. Such a technique leads to errors in itself. First, one surely should make distinction between the significance of the cavity which has invaded the dentin and which endangers the pulp, on the one hand, and on the other the defect which is equivocally carious and which one examiner may interpret as having had caries experience while another with equally good training may designate it as noncarious. It becomes necessary to distinguish between lesions on the basis of their relative extent, and as to whether or not the dentin has been obviously broached. Secondly, one should consider the variability of susceptibility of specific tooth areas to decay. The location of caries of itself is statistically of interest.

In setting up the technique of choice for the registration of caries incidence, extent or advance, the observer should choose the method best fitted to his specific objective. When considering caries as a community disorder, there is virtue in dealing with massed individuals, and deriving data which express the extent of caries within the population without necessary regard for the individual. However, one should hesitate to apply results so obtained to what may be expected in the individual subject. All studies of caries occurrence and advance reveal the wide range of values to be observed, even among individual subjects whose attributes are as similar as one can expect to find among different persons. If one wishes to portray the variability of spontaneous pattern of caries development and advance, he must deal with the records of individual subjects; or still better, with individual teeth. To estimate rates of advance, one should deal with serial records of the same sub-

ject. Even with the most meticulous technique, one may expect discrepancies to arise between serial examinations of the same patient's mouth, because of differences of judgment of the examiner from one examination to another.

The more one works in the field of clinical caries research, the more one realizes the limitations of accuracy attainable in the clinical estimation of caries advance. This report indicates that errors of judgment of notable degree may be introduced through a single prevalent practice: namely, the failure to give consideration to whether or not fillings have been placed prior to the study or are placed during its course. Various other errors are indigenous to clinical studies. Individually they serve to confuse the apparent course of caries in a specific mouth. Cumulatively they may lead to apparent changes of caries progression rates which are greater by far than the changes which are often attributed to the use of specific agencies for the modification of the pattern of caries advance. Insofar as most studies dealing with the control of caries through specific means have failed to take these commonplace variables into consideration, conclusions based on the data from such studies should be accepted only with reservation of judgment.

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EFFECT OF TEMPERATURE UPON THE DEXTRINIZATION OF STARCH BY SALIVARY ENZYMES

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TEMPERATURE is a very critical factor in determining the speed of starch breakdown under the action of saliva. That such a relationship exists is not surprising because of the known relationships between temperature and the speed of enzyme activity. Kneen's[1] work reflects the influence of temperatures on the action of malt diastase. The following data present a methodical study of the effect of temperatue on salivary amylase.

These findings are of practical importance for persons concerned with measuring enzymically-induced starch breakdown as it may relate to dental caries. A relationship between the speed of starch breakdown for the individual, (diastatic activity,[2] hydrolyzing time,[3, 4] dextrinizing time[5]) and his dental caries rate has been reported by several workers. Since this test may have a clinical as well as a theoretical value, any factor which affects the results should be studied. The temperature factor is so important that it will confuse the results of different workers if their tests are carried out at different temperatures.

The method used in this study is as follows: Six water baths were maintained at temperatures of 90° F., 80° F., 75° F., 70° F., 60° F., and 50° F. In each bath a test tube of 1 per cent KI and a test tube of 3 per cent H_2O_2 were inserted. The baths also contained empty testing tubes. Sufficient time was allowed for temperatures to equalize.

Pooled saliva from different individuals was collected with as little elapsed time as possible. The saliva was divided into six graduated test tubes. One per cent corn starch, in amount equal to the saliva, was added to the test tube containing saliva. The tube was immediately immersed in the appropriate water bath. A test aliquot was withdrawn at frequent intervals to determine the lengths of time for dextrinization. This determination is made as follows: 3 drops of saliva-starch mixture is withdrawn into one of the appropriate empty testing tubes; to this is added 3 drops of the 1 per cent KI from the appropriate water bath. After mixing by shaking, 5 drops of 3 per cent H_2O_2 are introduced from the appropriate water bath. Immediately after the addition of the last solution, color is read upon the mixing shake. If purple or blue color is seen the end point has not been attained. If brown is the only color seen on this mixing shake the end point has been reached. Yellow color is a further point beyond brown. The time is noted as elapsed minutes between original introduction of starch to saliva and this brown end point. This time is recorded in minutes as the dextrinizing time for the saliva-starch mixture from any given water bath.

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TIME VARIATIONS (IN MINUTES) IN A STARCH-AMYLASE REACTION OF DIFFERENT (F) TEMPERATURES

50°	60°	70°	75°	80°	90°
--	--	22	18	15	6
--	82	40	35	24	15
--	90	20	15	10	4
240	90	20	10	6	2
220	95	25	20	11	8
193	--	32	28	14	5
189	80	16	12	8	3
180	110	30	18	17	6
210	110	30	15	11	5
174	--	18	12	8	3
Mean					
200.9 min.	93.9 min.	25.3 min.	18.3 min.	12.4 min.	5.7 min.

Dextrinizing activity which requires 200 minutes at a temperature of 50° F. takes place in less than 6 minutes at a temperature of 90° F. In studying amyloclastic activity in relation to dental caries susceptibility, working temperature is a factor which must be controlled.

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A NEW TECHNIQUE FOR ORAL EXAMINATION OF RODENTS

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UP TO the present, one of the most serious handicaps in the use of rodents in investigation of oral diseases has been the lack of an easy and dependable method for routine oral examinations of the live animals. The small size of the teeth and the surrounding soft tissues and the posterior location of the molars make observation with the naked eye of questionable accuracy.

An oral examination can be performed under the dissection microscope if the animal is properly anesthetized. However, this procedure is time consuming and always carries the risk of premature sacrifice of the animal. The development of an easy and dependable method of examining living unanesthetized animals therefore seemed desirable. The technique about to be described is simple, quick, and noninjurious when proper experience is attained.

TECHNIQUE OF ANIMAL IMMOBILIZATION

Fig. 1 illustrates the device used in this technique and for descriptive purposes, individual parts have been named and labelled. The device is held in the right hand with the index finger lightly pressing down the distal end of the maxillary adjustment lever. The thumb rests on the horizontal part of the frame. The adjustment screw is only partially tightened to allow a rotating movement of the maxillary adjustment lever.

The hamster is grasped with the thumb and middle finger of the left hand and the loose skin of the neck is pulled backward. This procedure forces the animal's mouth open, separating the maxillary and mandibular incisors. The index finger rests on the animal's head.

The maxillary incisors are then introduced into the wire loop of the maxillary stabilizer with a forward rotation of the adjustment lever. (Fig. 2). The index finger of the left hand now rests on top of the adjustment lever to keep the animal in place. When the wire loop is properly seated, the nasal cross bar is lowered with the left index finger to lock the maxilla in a fixed position. The four fingers of the left hand slide behind the animal's head and are positioned in such a way that the head cannot be retracted from the wire loop.

The adjustment screw is only partially tightened and the adjustment lever is lowered with the right hand. The animal is manipulated with the left hand in such a way as to introduce the mandibular incisors into the opening on the mandibular stabilizer (Fig. 3). When this is achieved, the mouth is opened by a combined upward sliding motion and backward rotation of the maxillary adjustment lever. Then the adjustment screw is tightened with the left hand, while the fingers of the right hand temporarily rest on the animal's head to hold it in position.

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CONSTRUCTION OF DEVICE

In the construction of this device the materials used and the dimensions were as follows:

1. The frame is made of brass 1 inch wide with a median slit of 3/16 inch. Its vertical part is 3 inches long and the horizontal part 2 inches.
2. The maxillary adjustment lever is made of brass spring metal ½ inch wide and has a total length of 3½ inches. The mesial ½ inch is bent to parallel the vertical part of the frame.
3. The maxillary stabilizer is made of steel wire contoured around the adjustment lever to keep it in position.
4. The wire loop of the maxillary stabilizer is ⅜ inch wide and ½ inch high when the nasal crossbar is in an elevated position. For rats these dimensions are slightly larger.

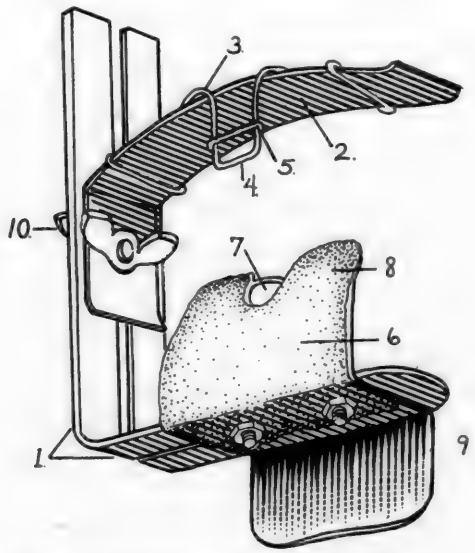

Fig. 1.—Device used for oral examination of laboratory animals.
Key: 1, frame
2, maxillary adjustment lever
3, maxillary stabilizer
4, wire loop of maxillary stabilizer
5, nasal crossbar
6, mandibular stabilizer
7, opening for the mandibular incisors
8, lateral wings of the mandibular stabilizer
9, body brace
10, adjustment screw

5. The nasal crossbar is so constructed that it slides up and down to increase or decrease the size of the wire loop. It has a snug fit to keep it stationary when the animal is positioned.
6. The mandibular stabilizer is made of aluminum and covered with serrated rubber on the outer surface. It is 1½ inches wide and continuous with the body brace (9). It is attached to the horizontal part of the frame with screws.

7. The opening for the mandibular incisors is located ½ inch above the frame, and the lateral wings (8) extend to a height of 1 inch. (If the device is to be used on the rat, the distance between the lateral wings must be increased to allow proper retraction.)
10. The adjustment screw holds the maxillary adjustment lever in position and accounts for the rotatory and sliding mobility of the latter.

With these materials and constructed according to the specifications the device is light, easy to handle, and with the animal in place, follows the shape of the hand.

TECHNIQUE OF EXAMINATION

A dissection microscope is employed in the examination and a magnification of (1×12) is sufficient to get a detailed picture of all the molars in one quadrant.

The animal is shifted to the operator's left hand with the fingers placed so that the head cannot be moved backward. For examination of the maxillary molars, the animal is positioned with these teeth nearly parallel to the microscope table. The cheek and masticatory muscles are retracted with a flat instrument in the right hand. The author finds that the use of one blade of a pair of slightly separated cotton pliers is very effective for this purpose. The same procedure is followed for the examination of both upper quadrants.

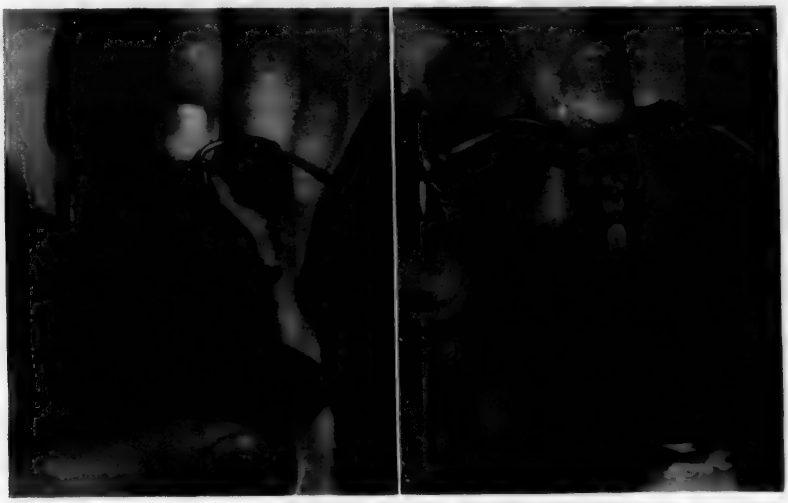

Fig. 2.—The maxillary incisors are introduced into the wire loop of the maxillary stabilizer.

Fig. 3.—When the wire loop is properly seated, the cross bar is lowered to lock the maxilla in a fixed position. Next the mandibular incisors are introduced into the opening on the mandibular stabilizer.

For examination of the lower left quadrant, the position of the animal is changed so that the mandibular molars are parallel to the microscope table. The animal is still held in the left hand. To examine the lower right quadrant, the animal must be transferred to the operator's right hand.

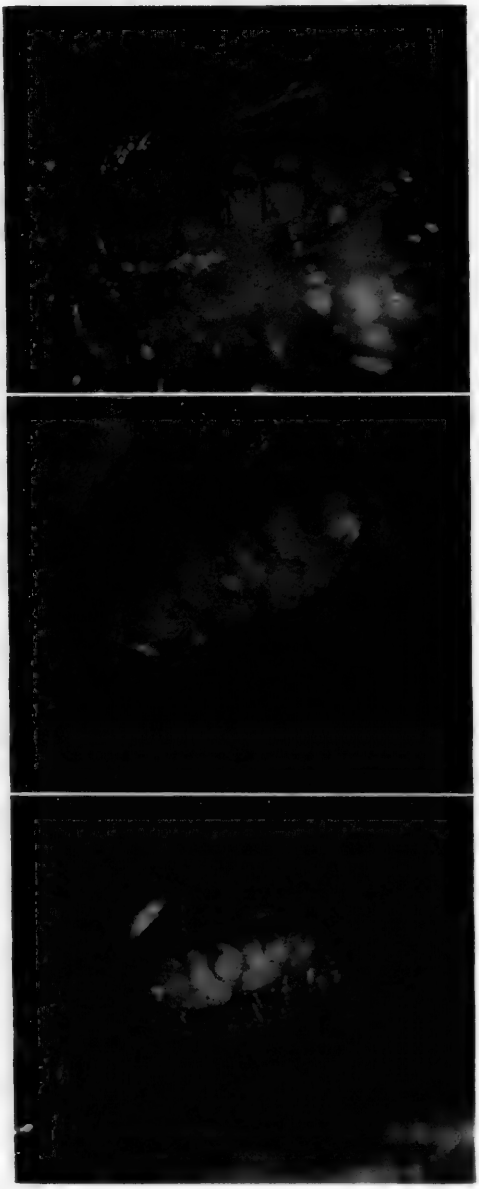

Fig. 4.—Photomicrographs of the maxillary molar teeth of live, unanaesthetized animals (Microscopic magnification, 1×12). *a*, Syrian Hamster. *b*, Rat, *c*, Mouse.

The main difficulty in observing the mandibular molars is the disposal of the tongue which obstructs the view of the teeth. This can be overcome in two ways. First, slightly separated cotton pliers may be inserted over the molars so as to push the tongue lingually and the cheek buccally. This method is the quickest and also entirely satisfactory if a visual examination is the only objective. Secondly, the tongue may be extruded and locked under the thumb on the lateral wing of the mandibular stabilizer. The serrated rubber covering on the mandibular stabilizer helps prevent the tongue from slipping back. Only slight pressure is needed to keep the tongue extruded when the animal is quiet. The second method is more desirable if an explorer is to be used in the examination.

Good light is essential for the examination and after having tried several methods, the author has found that two goose neck reading lamps with 100 watt bulbs, one on either side of the microscope, proved very satisfactory. No injurious effects have been observed from the light or heat.

Fig. 4*a*, *b*, and *c* are photomicrographs of the maxillary molars of a live, unanaesthetized hamster, rat, and mouse, respectively, and represent the field of vision with a microscopic magnification of (1×12).

One of the main difficulties before this technique is mastered is the exertion of the right amount of pressure by the thumb. If too much is applied, asphyxia results and immediate release is necessary. However, with prompt administration of artificial respiration, the animal is quickly revived. As a precaution against accidents, the operator should always observe the color of the oral mucous membrane during the examination and note the respiratory movements of the tongue. Impending asphyxia is generally preceded by violent muscular reaction with subsequent cyanosis and cessation of the respiratory movements of the tongue and epiglottis.

SUMMARY

1. A complete and reliable oral examination of laboratory animals can be achieved quickly without the use of anaesthetics and without sacrificing the animal.

2. The examination can be completed without touching the molar teeth or gingiva.

3. The technique is simple, quick, and noninjurious to the animal when proper experience is attained.

The author wishes to thank Dr. D. Afonsky of the Division of Dental Research, the University of Rochester for his helpful suggestions in the construction of the device and for taking all of the photographs.

PHYSICAL PROPERTIES OF DENTIN

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FOR some time there has been considerable interest directed toward the evaluation of dental restorations from the physical property standpoint. The dental profession has long recognized that the success of a restorative appliance is dependent not only on the satisfaction of biologic criteria but, in addition, on the capabilities of the restorative materials as well as the tooth structure to resist external displacements and internal deformations. For purposes of clarity, *external displacement* is defined as a relative movement of the restoration with respect to the tooth, while *internal deformation* is defined as the dimensional change of the restoration or tooth with respect to itself. These effects are governed by the laws of mechanics and the inherent physical properties of the materials involved; either of them can result in the failure of the restorative attempt.

These movements are caused by forces applied to the tooth and restoration which, in turn, may be manifestations of mastication, thermal effects within the mouth, setting change of the restorative materials, and insertion of the restoration.

Present-day restorative preparations are results of years of practical experience and investigation, and have been qualitatively designed for retention or for resistance to external displacement, as well as to restore functon to the member involved. The quantitative analysis of external displacement and internal deformation, however, has not been investigated to any great extent, largely due to a lack of knowledge of the physical properties of tooth structure and restorative materials under the conditions which exist in the mouth.

As an aid in solving the over-all problem of the effects of these dimensional changes, this study was directed toward the determination of the physical properties of dentin. G. V. Black[1] (1895) determined the crushing strength of .080 inch cubes of dentin, but because of his equipment limitations, was unable to determine the proportional limit or the modulus of elasticity. Subsequent to this date, no substantial work has been done on these properties.

The difficulties connected with this investigation were quite extensive due to the many existing variables and will be borne out by the ensuing discussion. In general, the preparation of test specimens and the testing of them were further complicated by the small sizes involved and the absence of engineering testing procedures for these sizes, in addition to the heterogeneity of the tooth structure itself. As a result of these difficulties, the initial work, which is reported in this paper, was the determination of the existence of all the variables involved, so that further investigation on the singular effect of these variables could be expedited. In addition, working values of the physical properties of dentin were desired in order to establish a basis for analyzing the movements as described previously.

This study was supported by a research grant from the Veterans Administration V-1001M-1543.

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SPECIMEN PREPARATION

First and second molar teeth were used for this study since they usually have relatively large amounts of dentin. These teeth were freshly extracted and were stored in water until time of test. The enamel was removed from the occlusal surface by grinding until the dentin was exposed. During this operation, the teeth were constantly dipped in water to prevent drying out and also to offset adverse temperature effects. They were then mounted in a steel ring filled with dental stone and the ring was placed in a lathe. Hollow drills were specially designed to be used for drilling out test samples. The design was such that water was in continual contact with the tooth. After this operation, the cylindrical specimens were placed in a special jig and the ends were finished to insure parallelism and flatness. They were then stored in water until time of test. The average size of these specimens was approximately 4.5 mm. long and 1.8 mm. in diameter.

TESTING PROCEDURE

The physical properties of dentin studied were the modulus of elasticity, proportional limit, and compressive strength, all of which can be determined from an applied stress and subsequent strain relationship for the material. By definition, stress is load or force per unit area and strain is deformation per unit length. As a consequence, the terms stress and load, strain and deformation, are often used synonymously since they are related simply by the geometry of the specimen.

The determination of strain presented a problem due to the small specimen size. A special loading device was made to allow for this measurement. Fig. 1 shows this device with a mounted specimen and the position of two Tuckerman Optical Strain Gauges. Eccentricity effects, which might enter into the strain measurements, were offset by the mounting of two gauges instead of one. The device consists of a rigid frame in which is mounted a stationary steel rod and a moveable one. The specimen was placed between the steel rods and load was applied through the upper moveable rod. By straddling the specimen, the strain gauges measured the deformation in both the specimen and that portion of the steel rods which lay within the gauge length. However, the deformation in the steel was negligible, for the loads applied. Therefore, the readings taken reflected only deformation in the dentin.

The entire assembly was then placed in a Tinius Olsen Universal Testing Machine and load was applied to the moveable steel rod. Strain readings were taken simultaneously. A stress-strain curve was plotted for each specimen, a sample of which is shown in Fig. 2. The points at which the three physical properties and their average values were determined by the tests conducted are also shown in this graph. By definition, the modulus of elasticity is the slope of the stress-strain curve within the elastic limit; the proportional limit is the stress beyond which stress is no longer proportional to strain; the compressive strength is the highest stress the material will withstand.

These properties define the inherent strength of the dentin and can be used quantitatively to predict its behavior under applied stress.

VARIABLES INVOLVED AND THEIR DETERMINATION

The main variables involved in this problem are indicated as follows and are accompanied by the measures employed to determine them:

1. Physiological differences in the teeth tested—
   Knoop Hardness Tests (500 Gm. load) used as control tests, as well as a stress-strain relationship, were made on each specimen.
2. Directional effects in the tooth structure—
   After the test, specimens were decalcified and the direction of the tubules relative to the axis of the cylindrical specimens was determined.
3. Rate of stress application—
   An attempt was made to keep this variable constant with the equipment available; however, variations did occur and the different rates of loading were calculated.
4. Length to diameter ratio of the specimens—
   This variable was kept fairly constant, except where it proved desirable to determine its effects on the physical property measurement.
5. Soundness of specimen—
   Specimens were checked visibly for defects or the presence of decay and the type of fracture was recorded.

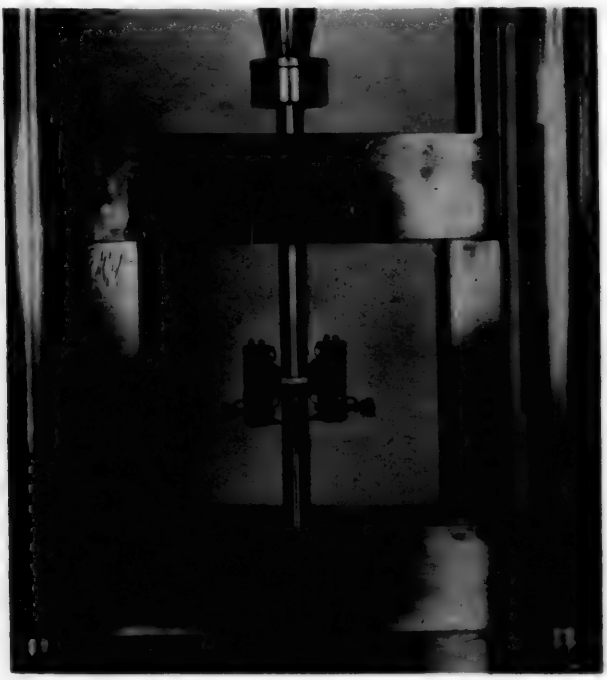

Fig. 1.—Dentin specimen mounted in a loading device showing the position of Tuckerman Strain Measuring Gauges. Entire assembly is shown between the loading platens of a Tinius Olsen Universal Testing Machine.

RESULTS

In Table I are shown the tabulated data for ten tests. The physical properties are recorded as well as the pertinent variables. As was mentioned, it

TABLE I
PHYSICAL PROPERTIES OF DENTIN AND INFLUENCING VARIABLES

SPECIMEN NUMBER	MODULUS OF ELASTICITY PSI	PROPORTIONAL LIMIT PSI	COMPRESSIVE STRENGTH PSI	KNOOP HARDNESS	TUBULE DIRECTION DEGREES	LOADING RATE PSI/MIN.	LENGTH TO DIAMETER RATIO
1	1,700,000	15,900	38,700	46	23	233,000	2.49
2	1,710,000	20,000	33,700	49	78	199,000	2.47
3	1,470,000	22,600	34,900	58	83	171,000	2.76
4	1,670,000	24,600	34,600	63	—	197,000	2.30
5	1 560,000	22,000	37,600	64	31	258 000	1.76
6	1,920,000	26 200	34,000	66	8	227,000	2.81
7	1,430,000	25 300	32,700	67	33	167,000	2.53
8	1,980,000	27,800	44,400	70	53	230,000	2.94
9	1,650,000	27,300	34,100	74	70	225,000	2.29
10	1.580,000	22,700	36,100	75	48	182,000	2.86
Average	1.670,000	23.400	36,100	63	47	209,000	2.52

was not found desirable, at this time, to isolate any of the variables to determine their singular effects on the physical properties. Instead, working values of modulus of elasticity, proportional limit, and compressive strength were initially deemed more important, and the averages of these ten tests were considered sufficient. A previous series of fourteen unpublished tests yielded an average modulus of elasticity of 1.66×10^6 psi. as compared to 1.67×10^6 psi. for

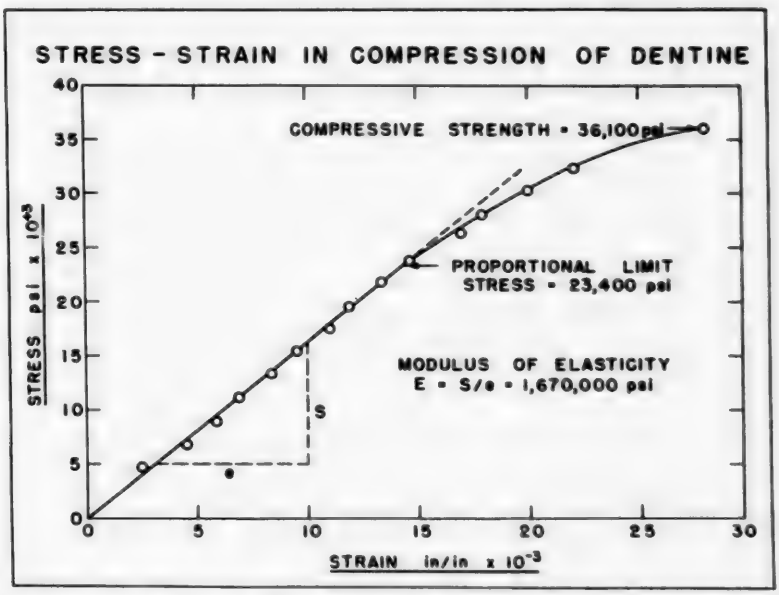

Fig. 2.—Stress-strain curve for dentin in compression indicating average useable values for the physical properties of this material.

these tests, and the work of G. V. Black[1] indicated an average compressive strength of 37,200 psi., compared to 36,100 psi. for these tests, thereby substantiating their validity. Inspection of the data indicates that all the variables men-

tioned are influential to some degree. Although the deviations of the physical property values from their averages may be as high as 20 per cent, it is important to realize that these are not random or experimental errors. These deviations can be correlated to a deviation of one or more variables from their average values, such that over-average, average, or under-average physical properties can be attributed to one or more over-average, average, or under-average variables. Specimens 3 and 8 were taken from the same tooth and, upon consideration of their Knoop hardness values, the results indicate that the physical properties of dentin may vary depending on the place from which the specimen was removed. This factor further validates the usage of an average physical property value since it would be most difficult to get a true value for any one tooth.

CONCLUSIONS

The following conclusions were drawn from this study:

1. The physical properties of dentin are influenced by the following variables:
   a. Physiological differences in teeth.
   b. Directional effects in the tooth structure.
   c. Rate of stress application.
   d. Length to diameter ratio of the test specimen.
   e. Soundness of test specimen.
2. The averages of the physical properties of dentin are as follows, and represent usable values for the analysis of stresses and subsequent movements in restorations and teeth:
   a. Modulus of elasticity—1.67×10^6 psi.
   b. Proportional Limit—23,400 psi.
   c. Compressive Strength—36,100 psi.
3. On the basis of Knoop hardness tests, the physical properties of dentin may vary within the same tooth, indicating the difficulty in obtaining true values for these properties.

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CRYSTALLOGRAPHIC INVESTIGATION OF TRICALCIUM PHOSPHATE HYDRATE

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THE existence of tricalcium phosphate hydrate, or α-tricalcium phosphate as it is sometimes called, is a problem which concerns workers in many fields. It has been suggested by Dallemagne and his co-workers[1, 2] that this substance, admixed with magnesium and calcium carbonate, is the main inorganic constituent of bone. However, chemists, biologists, and mineralogists, as well as scientists in other branches of research, cannot agree as to whether this substance is a real chemical entity or is merely a defective or physically modified hydroxy-apatite. The main problem has been the inability of the experimentalist to make exact x-ray and optical measurements on this material because of the extremely small size of the crystals of tricalcium phosphate hydrate found in natural or laboratory samples.

There are two main structural concepts of this precipitated calcium phosphate in which the formula $Ca:PO_4$ ratio is 1.50 as opposed to 1.67 for hydroxy-apatite. One viewpoint, held by Dallemagne, Brasseur, and Melon,[3] is that since the x-ray patterns of these substances are quite similar but their optical and chemical properties are different, tricalcium phosphate hydrate may be thought of as a calcium-deficient hydroxy-apatite, $Ca_9(PO_4)_6H_2(OH)_2$, with two hydrogen ions in place of the missing calcium. On the other hand, Bale, LeFevre, and Hodge[4] maintained that the tricalcium phosphate hydrate is merely hydroxy-apatite with phosphate ions adsorbed in sufficient quantities to reduce the $Ca:PO_4$ ratio to the 1.50 value.†

In order to characterize tricalcium phosphate hydrate conclusively, it is necessary to enlarge the crystals so that precise crystallographic measurements can be made. Then, by combining these x-ray and optical data with the chemical data, it may be possible to reach a reasonable conclusion.

CRYSTAL GROWTH

Commercial tricalcium phosphates were chosen as specimens for the crystal growth experiments. These substances had an original particle size of about 500 angstrom units. As cited by Hodge, LeFevre, and Bale,[5] the composition of commercial tertiary calcium phosphates is found to vary considerably. Therefore, precautions were taken to choose only those phosphates which had a $Ca:PO_4$ ratio as close as possible to 1.50, the theoretical ratio in α-tricalcium phosphate.

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†For a more complete review of the theories concerning tricalcium phosphate hydrate, see the article by Dallemagne, Brasseur and Melon.[3]

TABLE I.

TYPICAL RESULTS OF CHEMICAL AND PHYSICAL ANALYSES OF SAMPLES OF TRICALCIUM PHOSPHATE HYDRATE AND HYDROXY-APATITE

SAMPLE NO.	BOMB TIME AND TEMPERATURE	Ca:PO_4 RATIO	REFRACTIVE INDEX AVERAGE	CRYSTAL SIZE (MICRONS)	LATTICE CONSTANTS a	LATTICE CONSTANTS c	DENSITY	X-RAY AFTER 900° C.
S-1	Untreated TCPH*	1.495	1.60	0.05	9.43	6.88	2.89	β TCP
S-4	2 hours 300° C.	----	1.60	0.1	9.42	6.88	----	β TCP
S-8	2 days 300° C.	1.492	1.60	0.5	9.42	6.87	2.89	β TCP
S-10	7 days 300° C.	1.508	1.60	1.0	9.43	6.88	----	β TCP
S-11	2 days 300° C.	----	1.60	0.5	9.44	6.88	----	β TCP
S-12	7 days 200° C.	----	1.60	0.1	9.42	6.88	----	β TCP
S-15	(Hydroxy-apatite)	1.642	1.64	0.5	9.45	6.89	3.13†	OH-AP
S-29	(OH-Ap + H_3PO_4) 7 days 300° C.	----	1.61	0.5	9.45	6.89	2.96	β TCP + OH-Ap (minor amount)

*Tricalcium phosphate hydrate.

†This density calculated from x-ray data; others observed.

The material was then treated in a hydrothermal bomb according to the method of Morey and Ingerson.[6] A charge of about 2 Gm. of the original, poorly crystallized material was introduced into the platinum-lined chamber of the bomb (roughly 20 ml. in volume). This was then covered with about 4 ml. of distilled water, the bomb was sealed, using a platinum gasket, and was inserted into a furnace which was held at the desired temperature for a given time. The temperatures used ranged from 250 to 450° C., resulting in hydrothermal pressures that attained approximately 2,000 psi. The experiments lasted from a few hours to a few weeks.

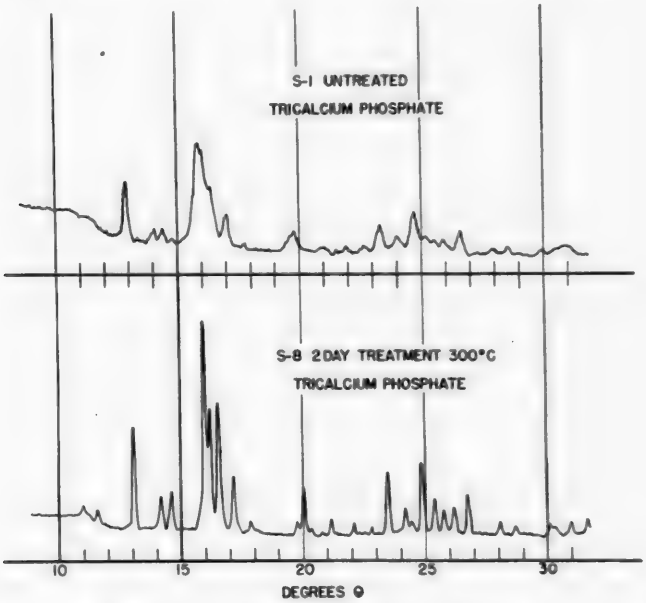

Fig. 1.—X-ray powder diagrams of untreated and treated tricalcium phosphate hydrate.

The results of the crystal growth were followed by x-ray diffraction and electron and optical microscopy. In Fig. 1 the improvement of crystallinity is shown by the sharpening of the x-ray pattern of the sample when treated in a bomb at 300° C. for two days. The same samples are shown in Figs. 2 and 3, as seen under the electron microscope, the former being the untreated, the latter the treated sample. Fig. 4 shows the same sample treated at 300° C. for one week. It is of interest to note that the crystallite size of the original unimproved sample, as calculated by the x-ray line broadening (Fig. 1) according to the method outlined by Biscoe and Warren,[7] came to an average value of 500 angstroms. This checks very well with the finest particles seen in the electron micrograph of this substance (Fig. 2). Since the particle size and the crystallite size are of about the same dimensions, this leads to the conclusion that the ultimate particles of the tricalcium phosphate hydrate observed are single crystals.

There has been no attempt in this paper to report on a systematic study of the hydrothermal growth of the tricalcium phosphate hydrate crystals. The experimental conditions mentioned herein were chosen arbitrarily since they suited the available equipment and furnished us with crystals large enough for making accurate x-ray and optical measurements.

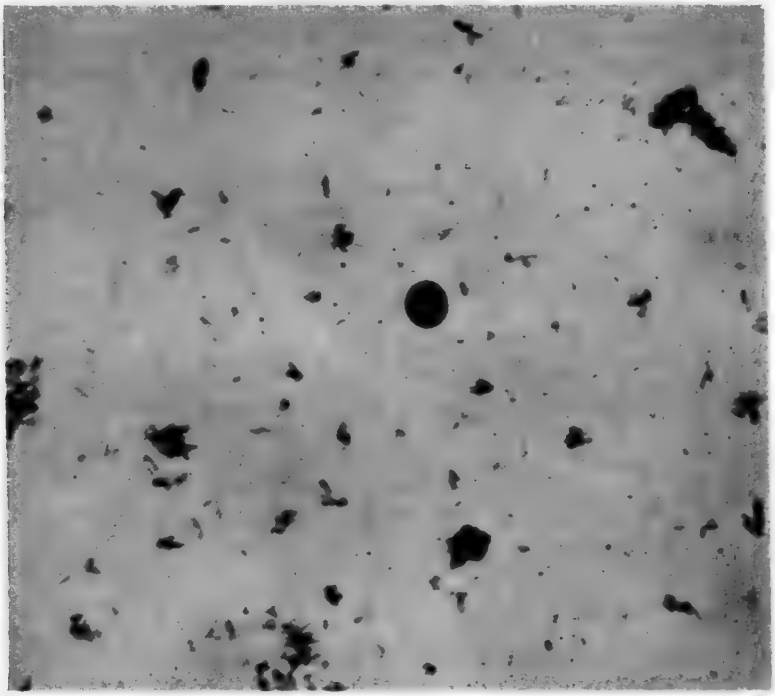

Fig. 2.—Electron micrograph of untreated tricalcium phosphate hydrate. Magnification 20,000 diameters. The spherical polystyrene particle in center of picture is about ¼ micron.

The results of a few representative crystallization experiments can be seen in Column 5 of Table I. This table contains data on only those samples made from Baker & Adamson C. P. tribasic calcium phosphate (Serial number 3772). It was found that the minimum treatment necessary to sharpen the x-ray diffraction pattern (i.e., to grow the crystals larger than about 0.1 μ) was a 2 hour digestion in the hydrothermal bomb at 300° C. with a resulting internal pressure of approximately 2,000 psi. As the same treatment was continued for a week, the crystals grew to a length of about 2 to 3 μ (Fig. 4). This size seems to be the practical maximum that can be attained under these experimental conditions, since doubling the time of digestion produced no measurable increase in particle size.

ANALYSIS

The original, poorly crystallized samples and a few of the improved samples were analyzed for calcium and phosphate, and the $Ca:PO_4$ ratios were calcu-

lated. The methods used are described by Kolthoff and Sandell.[8] The calcium was precipitated as the oxalate and ignited to constant weight as CaO, while the phosphate was determined by precipitating it as magnesium ammonium phosphate and igniting to constant weight as $Mg_2P_2O_7$. The results are given as the formula $Ca:PO_4$ ratios in Column 3 of Table I.

In every hydrothermal experiment the residual bomb water was analyzed by microchemical qualitative methods for the presence of calcium and/or phosphate ions. In general these methods depend upon the addition of a suitable reagent to a drop of the unknown solution which has been placed on a slide mounted on the stage of a petrographic microscope. The separation of a solid phase which possesses crystalline properties peculiar to the ion being sought is verified by microscope. In the case of the calcium an attempt was made to precipitate $Ca(C_2O_4)\cdot 3HO$ and, in the case of the phosphorus, to precipitate the complex compound formed between the phosphate ion and silver nitrate.*

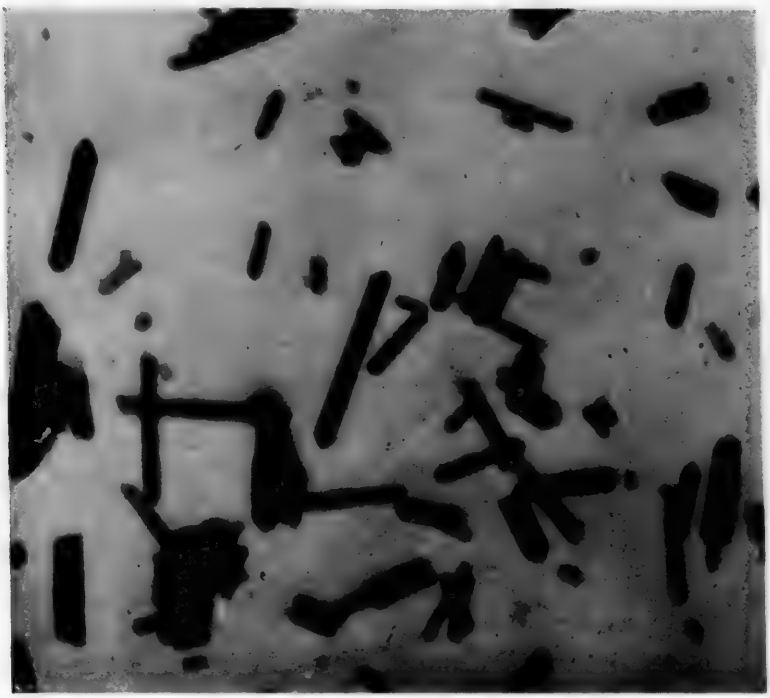

Fig. 3.—Electron micrograph of tricalcium phosphate hydrate treated for two days at 300° C. in a hydrothermal bomb. Magnification 20,000 diameters.

There was no trace of calcium or phosphate found in all of the microanalyses. It was then decided to abandon the complete chemical analyses since it could be assumed that there was not change in the original $Ca:PO_4$ ratio if there was no appearance of these ions in the water phase. In general it can be

*For details of these methods refer to pages 114 and 336, respectively, of Chamot and Mason's Handbook of Chemical Microscopy, Volume II.[9]

concluded from the analytical results that the original substance had a $Ca:PO_4$ ratio equivalent to the theoretical ratio of tricalcium phosphate hydrate and that no change occurred during the hydrothermal treatment of this sample.

IGNITION OF PHOSPHATES

It is well established that calcium phosphates which display the x-ray diffraction pattern of hydroxy-apatite and have a $Ca:PO_4$ ratio of 1.50 will give an x-ray diffraction pattern of β-tricalcium phosphate when ignited at 900° C.[5, 10] On the other hand, it is also known that a calcium phosphate giving the hydroxy-apatite x-ray pattern and having a ratio of 1.67 will not change its x-ray pattern upon ignition at the same temperature.[5, 10] This has been used as a method of differentiating between tricalcium phosphate hydrate and hydroxy-apatite. Some samples of tricalcium phosphate hydrate and a hydroxy-apatite were subjected to such an ignition at 900° C. for at least eight hours, and the resulting x-ray diffraction patterns are tabulated in Column 9 of Table I.

Fig. 4.—Electron micrograph of tricalcium phosphate hydrate treated for seven days at 300° C. in a hydrothermal bomb. Magnification 20,000 diameters.

X-RAY DIFFRACTION AND OPTICAL STUDIES

All of the calcium phosphates studied were examined as powders in a Geiger counter x-ray spectrometer using $CuK\alpha$ radiation. A few of the typical results can be seen in Figs. 1 and 5. The principal observation made on the patterns of

tricalcium phosphate hydrate is that they are very similar to one another regardless of the conditions under which they were prepared. In fact, with the exception of very slight differences in cell dimensions (see Table I, Columns 6 and 7), the various tricalcium phosphate patterns are identical. The x-ray powder pattern of a typical sample of "improved" tricalcium phosphate hydrate (S-8) was carefully compared with the powder pattern of hydroxy-apatite* (S-15). These patterns are shown in Fig. 5 and Table II.

All samples were examined with the petrographic microscope. The average indices of refraction obtained by the Becke line method are listed in Column 4 of Table I.

DENSITY MEASUREMENTS

The densities of some of the samples were determined by a modification of the ordinary pienometer method usually applied to liquids. After the pienometer was calibrated with distilled water, a quantity of the powdered sample to be studied was added to the dry, weighed pienometer and the entire system was weighed again. The pienometer was then half filled with water and subjected to a vacuum treatment for five to ten minutes in order to rid the sample of any air which might lead to erroneous density measurements. The pienometer was then filled to the mark and weighed at 20° C. The density was calculated according to the method outlined by Hidnert and Jeffer[11] using the following equation:

$$D = \frac{dS}{S + P + Vd - W'}$$

where D = density of the powder
d = density of water at 20° C
S = weight of the powder sample
P = weight of the dry pienometer
V = internal volume of pienometer
W' = total weight of the pienometer, powder and water.

The results of these measurements may be seen in Column 8 of Table I.

ADSORBED PHOSPHATE IONS

Bale, LeFevre, and Hodge[4] gave the reaction (unbalanced) in which tricalcium phosphate hydrate transforms to β-tricalcium phosphate upon ignition at 900° C as follows:

$$3\ Ca_9(PO_4)_6Ca(OH)_2 + (PO_4)_2^{-3} \rightarrow 10\ Ca_3(PO_4)_2$$

This viewpoint stems from their theory that tricalcium phosphate hydrate is indeed hydroxy-apatite with adsorbed phosphate ions.

A series of experiments was performed to test the above reaction. A charge of 2 Gm. of hydroxy-apatite was added to the hydrothermal bomb and covered with 4 ml. of a dilute solution of orthophosphoric acid, the concentration of which was adjusted to change the total $Ca:PO_4$ ratio of the charge (solid plus liquid) from 1.67 to 1.50. This was then sealed as usual and heated for seven

*Sample obtained from the National Institute of Dental Research. This sample is not pure hydroxy-apatite, as shown by its analysis (Table I); however, there were no spurious lines in the diffraction pattern and so it was chosen as a standard.

TABLE II.

COMPARISON OF X-RAY DIFFRACTION DATA OF HYDROXY-APATITE AND TRICALCIUM PHOSPHATE HYDRATE

	HYDROXY-APATITE		TRICALCIUM PHOSPHATE HYDRATE			HYDROXY-APATITE		TRICALCIUM PHOSPHATE HYDRATE	
hkl	DEGREES θ	RELATIVE INTENSITY*	DEGREES θ	RELATIVE INTENSITY*	hkl	DEGREES θ	RELATIVE INTENSITY*	DEGREES θ	RELATIVE INTENSITY*
200	10.80	16.5	10.85	9.95	302	20.90	7.9	21.05	9.5
020					032				
111	11.34	9.2	11.45	8.5	400	21.92	6.6	21.98	7.8
002	12.89	35.6	12.95	48.5	040				
102	14.00	9.7	14.05	16.6	203	22.62	6.0	22.70	5.9
012					023				
120	14.35	17.0	14.50	18.95	222	23.25	30.1	23.40	32.0
210					312	23.98	16.0	24.10	14.7
121	15.80	100.00	15.92	100.00	132				
211					230	24.23	6.8	24.35	8.3
112	16.05	57.8	16.11	58.0	320				
300	16.35	64.1	16.50	61.7	213	24.70	33.8	24.80	35.5
030					123				
202	16.95	26.2	17.05	27.2	321	25.20	17.3	25.30	19.0
022					231				
301	17.65	9.2	17.75	7.1	410	25.55	12.8	25.65	13.3
031					140				
130	19.55	11.3	19.65	9.0	402	25.95	13.6	26.08	14.7
310					042				
221	19.80	24.9	19.90	23.6	004	26.60	17.1	26.65	21.5
103	20.35	2.6	20.25	4.5	500	27.85	7.9	27.97	7.6
013					050				
311	20.55	2.4	20.65	3.6	133	28.49	4.7	28.58	5.2
131					313				

*Height of peak on spectrometer record.

days at 300° C. The resultant sample was examined by x-ray and optical methods and part of the sample was ignited for 12 hours at 900° C. The results, as seen in Table I (Sample S-29) show that by adding phosphate ion to hydroxy-apatite and igniting it at 900° C, a β-tricalcium phosphate structure can be achieved. It should be noted that the addition of the phosphate ion has lowered the index of refraction from 1.64 to 1.61, the latter figure resembling the index of refraction of the chemically precipitated samples of tricalcium phosphate hydrate.

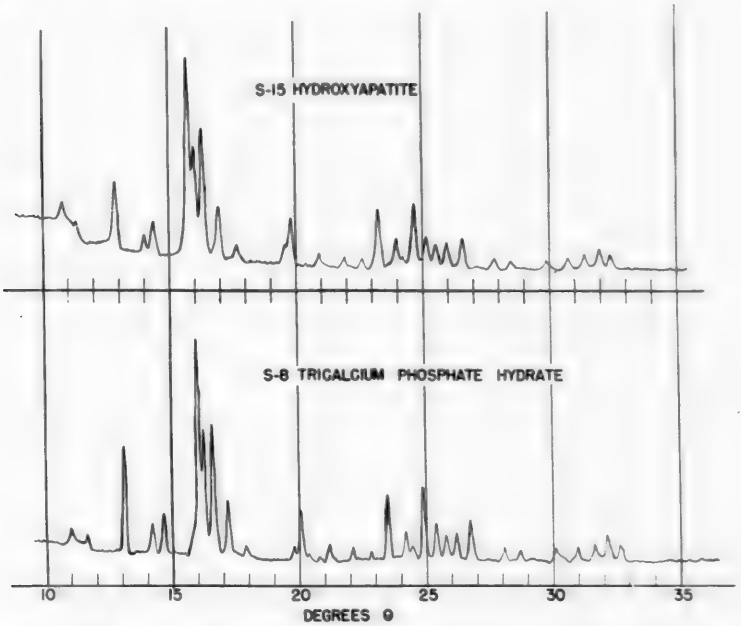

Fig. 5.—Comparison of the x-ray powder diagrams of hydroxy-apatite and a typical tricalcium phosphate hydrate.

CONCLUSIONS

From the analytical data obtained it might be concluded that tricalcium phosphate hydrate is distinctly different from hydroxy-apatite. However, their x-ray diffraction patterns are very similar, as shown in Fig. 5 and Table II. The small difference in the *a* axis might be explained by the fact that this axis in the "hydrate" crystal was not developed beyond 0.1 to 0.2 μ and such mal-developed axes have been known to give erroneous axial lengths.[12] There are also some differences in the intensities which remain unexplained at this point.

In general a structure must appear in some periodic arrangement whose size is of the order of 100 angstroms or greater in order to be detected by x-ray diffraction. It is conceivable that some substance or substances may be present in the tricalcium phosphate hydrate in large enough amounts to alter the $Ca:PO_4$ ratio, the index of refraction, the density, and the ignition properties without being in a developed, crystalline state which would appear in the x-ray data.

To correlate the analytical results with the above x-ray diffraction data one may assume some sort of phosphate ion adsorption or occlusion in the amount needed to change the $Ca:PO_4$ ratio of hydroxy-apatite from 1.67 to 1.50. If this apparent excess of phosphate, 6 per cent by weight, were present in a separate crystalline phase, it would be detected by x-ray diffraction, assuming of course the crystallites were larger than about 100 angstrom units.

The x-ray diffraction data, which show very little difference between hydroxy-apatite and tricalcium phosphate hydrate, could also be interpreted to show that the Dallemagne and Brasseur[3] formulation $Ca_9 (PO_4)_6 H_2 (OH)_2$ might be true. This configuration theoretically would give a pattern quite similar to the hydroxy-apatite. However, if the density of this substance is calculated from the unit cell volume, obtained from the x-ray data, and the molecular weight suggested by the above formula, it is found to be 3.03 as opposed to an observed value of 2.89. If the average index of refraction is also calculated using the Gladstone and Dale method,[13] the theoretical index is found to be 1.65, while the observed index is 1.60. If the hydrate is assumed to be a monohydrate instead of the proposed dihydrate, the theoretical index of refraction is found to be 1.64, which also does not agree with the observed value. To reconcile the density and index of refraction data with the x-ray diffraction data it may be assumed that some sort of diluent which fails to give a diffraction pattern is adsorbed or occluded in the hydroxy-apatite crystals, lowering the density and index of refraction by the amounts shown.

It is well to add that the method of Gladstone and Dale is only approximate and does not always give exact results. However, it is felt that when we compare similar compounds as above, the method is sufficiently accurate for our purposes. Thus we can state that any of the suggested formulas for tricalcium phosphate hydrate would give indices of refraction almost coincident with that of hydroxy-apatite.

A complete analysis of tricalcium phosphate hydrate shows that it contains anywhere from 1.15 to 7.30 per cent of volatile water.[5] The above evidence does not completely explain the existence of this excess water in these tricalcium phosphate hydrate crystals. It is strongly indicated that this water is not contained in the structure. One can guess that it is surface adsorbed water. There is some evidence from preliminary work done by the authors that when enlarged tricalcium phosphate hydrate crystals are subjected to differential thermal analysis, the curves are quite dissimilar to those obtained from the fine particle material. This lends support to the hypothesis of water adsorption since the enlarged particles could not accommodate such a large excess of water on the surface.

A systematic study of the addition of phosphate to hydroxy-apatite is still under way. So far our experiments have shown that by the addition of phosphoric acid to hydroxy-apatite a well crystallized product can be obtained which satisfies the tricalcium phosphate hydrate formula. This product has a density and a refractive index different from those calculated on the basis of an apatite of the composition $Ca_9 H_2 (PO_4)_6 (OH)_2$, while the x-ray diffraction pattern is clearly very similar to that of hydroxy-apatite.

There has been some question raised as to whether the improved crystals of tricalcium phosphate hydrate are the same as the original material. Since they analyze the same and no phosphate or calcium was found in the residual bomb water, and since no amount of bomb treatment, ranging from two hours to one week, changed the index of refraction, the density, or the x-ray pattern, it is clear that there has been no chemical or crystallographic change during this hydrothermal growth.

The exact nature and placement of the physically admixed ions, which according to this hypothesis make the hydroxy-apatite appear to be tricalcium phosphate hydrate, can only be speculated upon. Surface adsorption of phosphate ions cannot be the entire effect. In the 3 μ crystals of the improved tricalcium phosphate hydrate it would be impossible for 6 per cent by weight of the entire crystal to be phosphate ions adsorbed in any reasonable number of layers on the crystal surfaces. This lends emphasis to the explanation that the bulk of the phosphate diluent is occluded in the submicroscopic faults of the hydroxy-apatite crystal. We measure a density of 2.89 on these tricalcium phosphate hydrate crystals, which differs from the theoretical value of 3.13 for hydroxy-apatite. Considering the 6 per cent occlusion as phosphate ion, we cannot completely justify this low density by this factor alone. Thus, there must be a small amount of empty space in the crystal which, coupled with the lower density of the phosphate ions as compared to that of hydroxy-apatite, will combine to give the observed density. Apparently the phosphate ions (and the hydrogen ions associated with them for electrical neutrality) would deposit imperfectly in these entrapped surfaces during crystal growth so that there would be empty spaces remaining, thus accounting for the drop in density mentioned above. The results reported in this paper would not indicate whether the occluded ions were PO_4^{-3} HPO_4^{-2}, or some other ion involving phosphorus, but regardless of the exact nature of the ion, the principle of occlusion would still be the same and there would still be 0.67 H_3PO_4 for every hydroxy-apatite molecule to form one molecule of so-called tricalcium phosphate hydrate. Thus, to change the view of Hendricks and Hill[15] very slightly, the formula for tricalcium phosphate hydrate would be $Ca_{10}(PO_4)_6(OH)_2 \cdot$ occluded 0.67 H_3PO_4.

There are precedents for thinking in terms of imperfections in crystals. Addink[14] has shown that the density of certain crystals as calculated from very accurate x-ray data is found to be higher than the carefully measured density. It should be noted that these imperfections were of the order of magnitude of less than 1 per cent, much lower than the above 6 per cent. The mode of occlusion of excess phosphate ions in hydroxy-apatite may be similar to the carbonate occlusion suggested by Hendricks and Hill[15] for the carbonate apatites.

The higher content of phosphate in the apatite of bone, cementum, and dentin as compared with enamel indicates that a mechanism may be active which permits the occlusion of these ions in greater amounts in bone. Sobel, Hanok, Kirschner, and Fankuchen[16] have shown that the PO_4:CO_3 ratio of

blood serum bears a direct relationship to the PO_4:CO_3 ratio of enamel and dentin. This may indicate that the amounts of inclusions in the deposited apatites depend on the individual concentration of these ions in the serum.

SUMMARY

1. Crystals of so-called tricalcium phosphate hydrate were enlarged in a hydrothermal bomb from an average length of 500 angstrom units to about 3 μ without changing their Ca:PO_4 ratio, density, refractive index, and the spacings of their x-ray pattern.

2. The x-ray diffraction powder patterns of tricalcium phosphate hydrate and hydroxy-apatite were found to be quite similar.

3. The differences between hydroxy-apatite and tricalcium phosphate hydrate insofar as density, index of refraction, and results of ignition at 900° C. are concerned can possibly be explained by postulating a 6 per cent phosphate ion inclusion in the entrapped surfaces of the hydroxy apatite crystal.

4. We suggest that a possible formula for tricalcium phosphate-hydrate is $Ca_{10}(PO_4)_6(OH)_2$·occluded 0.67 H_3PO_4.

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THE STAINING OF NERVE FIBERS IN TEETH

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DESPITE the clinical evidence that the dentin is sensitive there remains wide controversy as to whether or not nerve elements are themselves responsible for this sensitivity. The older literature was well covered by Berkelbach,[1] Kronfeld,[2] and Brashear[3] and, recently, Bernick[4] has also discussed this question so there is no need for a complete review. Briefly, however, the reports on innervation of the dentin fall into three groups: some have claimed that the dentin is nonnervous (Hopewell-Smith,[5] Tiegs,[6] and Orban[7]); others that it contains a few nerve fibers (Wasserman,[8] Bernick[4] and Lewinsky and Stewart[9]); and still others apparently believe that the dentin is more richly innervated than even the finger tips (Tojoda,[10] Münch,[11] Mummery,[12] and Berkelbach[1]). Two factors are chiefly responsible for this controversy. The first of these is the relative difficulty of silver impregnation methods, and the second is the nonspecific nature of many of these methods for the staining of nerve fibers. In the case of the tooth the dentinal tubules and the connective tissue of the pulp are often darkly or erratically stained making it difficult either to trace or to differentiate nerve fibers.

Since published accounts concerning the innervation of the tooth have been limited to the use of only a few techniques, it was thought that a wide survey of possible methods might reveal one which gave comparatively complete impregnation of nerve fibers with not too much impregnation of nonnervous structures. Since block methods and frozen section methods are not flexible, only "on-the-slide" techniques were considered.

Several fixatives were tried, including 10 per cent formalin, formalin chloral hydrate mixture, Bodian's method No. 2,[13] 95 per cent alcohol fixation, Bouin's and a modified Bouin's solution. The following techniques were applied after these fixatives and gave unfavorable results—Bodian,[13] Davenport,[14] Palmgren,[15] and Pearson and Whitlock.[16] However, the silver nitrate methods of Romanes[17] and Ungewitter[18] gave very satisfactory results. The present report concerns the use of these last two methods with modifications which have given consistent results and convincing differentiation of nerve fibers in the pulp and the dentin of both human and rat teeth. An account of unsuccessful methods need not be presented.

MATERIAL AND METHODS

Freshly extracted noncarious adult human teeth were fixed in either formalin chloral hydrate solution (formaldehyde [37 per cent] 10 c.c., chloral hydrate 10 Gm., distilled water 90 c.c.) or modified Bouin's (saturated aqueous picric

Demonstration of some of this material was presented at the Meeting of the American Association of Anatomists, Detroit, Mich., on March 21, 1951; also at the American Dental Association Meeting, Washington, D. C., Oct. 15, 1951.

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acid 75 c.c., formaldehyde [37 per cent] 25 c.c. and trichloracetic acid 1 Gm.). Matured rat jaws were fixed overnight in the formalin chloral hydrate fixative. After fixation, decalcification with either formic acid sodium citrate mixture (Evans and Krajian[19]) or 5 per cent trichloracetic acid in 50 per cent ethyl alcohol proved satisfactory. After slow dehydration, tissue was embedded in paraffin and sectioned serially at 10 or 15 microns. Since the silver nitrate techniques of Romanes[17] and Ungewitter[18] are recent and hence not well known and since some modification was necessary for their use on teeth the various steps are presented. It was found essential to mordant the sections before staining by Romanes' method[17] in a 0.5 per cent cupric nitrate overnight in a 37° C. oven. This mordanting diminished background staining and intensified the staining of the nerve processes.

Method I.—

1. Fix the tissue in 10 per cent formalin with 10 per cent chloral hydrate for one to three days.

2. Wash in running tap water for 24 hours or overnight.

3. Decalcify in either equal parts of 20 per cent sodium citrate and 50 per cent formic acid or in 5 per cent trichloracetic acid in 50 per cent alcohol.

4. After tissue is completely decalcified wash in running tap water for 24 hours.

5. Dehydrate slowly using changes of 30 per cent, 50 per cent and 70 per cent ethyl alcohol for 24 hours in each; then into solutions of combinations of 95 per cent ethyl and n-butyl alcohols with final dehydration in n-butyl alcohol for two to three days.

6. Infiltrate with 56 to 58 degree paraffin for 12 to 24 hours.

7. Section at 10 or 15 microns.

8. Remove paraffin with xylol and hydrate through graded ethyl alcohols to distilled water.

9. Place in 0.5 per cent aqueous cupric nitrate (Baker's crystals, c.p.) solution overnight in 37 degree C. oven.

10. Rinse through several changes of distilled water.

11. From here on follow Romanes' silver chloride method[17] which is as follows:

12. Place overnight in the following solution in 56 degree C. oven:

Distilled water	50.0 ml.
0.1 per cent silver nitrate	2.9 ml.
0.1 per cent sodium chloride	1.0 ml.
1.0 per cent ammonia	16 drops (0.7 c.c.)

13. After incubation, the sections, now light brown in color, are rinsed in distilled water and developed for 5 min. in:

Hydroquinone	1.0 Gm.
Sodium sulphite, anhyd.	5.0 Gm.
Distilled water	100.0 ml.

14. Wash well and take through distilled water into 0.2 per cent gold chloride for 10 minutes.

Fig. 1.

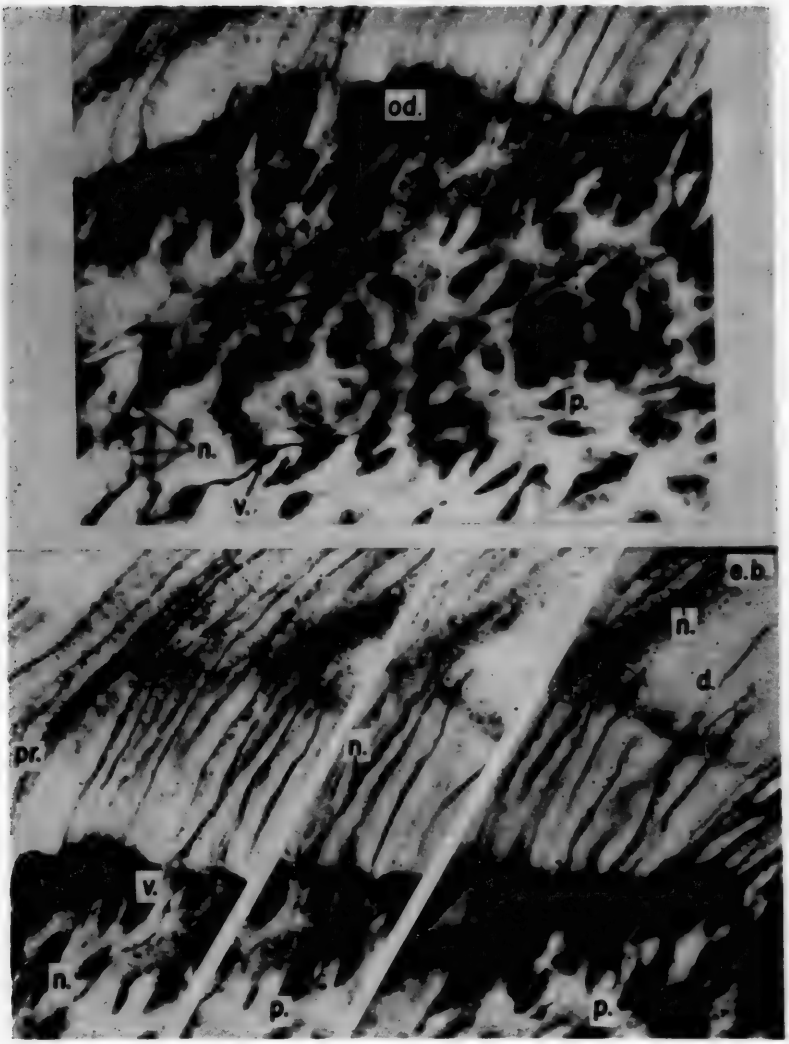

Fig. 2. Fig. 3. Fig. 4.

Figs. 1, 2, 3, and 4.—Longitudinal section through molar of rat. Method I. Sections show continuity of nerve fiber starting in pulp (Fig. 1), crossing the odontoblastic layer (Fig. 2), passing through the predentin (Fig. 3), and extending into the dentin ending in an end bulb (Fig. 4). *n.*, nerve; *pr.*, predentin; *d.*, dentin; *p.*, pulp; *v.*, varicosity; *e.b.*, end bulb.

15. Wash in distilled water for two minutes.

16. Place in 2 per cent oxalic acid until the nerves are clearly defined but for not more than 3 minutes.

17. Wash and place in 5.0 per cent sodium thiosulphate for 3 to 5 minutes.

18. Wash, dehydrate, clear and cover.

Method II.—

1. Fix tissue in the following solution for 3 days or longer:

Sat. aq. picric acid	75.0 c.c.
Formalin (37 per cent)	25.0 c.c.
Trichloracetic acid	1.0 Gm.

2. Follow Method I steps 2, 3, 4, 5, 6 and 7.

3. From here on follow Ungewitter's urea silver nitrate method[18] which is as described below:

4. Remove paraffin with xylol and run sections through absolute and 95 per cent alcohol to 80 per cent alcohol.

5. Immerse slides directly from the 80 per cent alcohol for 60 to 90 minutes at 50 to 60 degree C. (place in paraffin oven) in the following solution:

1 per cent aqueous silver nitrate	100.0 ml.
Urea, U.S.P.	20-30 Gm.
1 Gm. mercuric cyanide and 1 Gm. picric acid in 100 ml. distilled water	1 to 3 drops

6. Rinse quickly in 2 changes of distilled water.

7. Reduce for 3 to 5 minutes at 25 to 30 degree C. (room temperature) in a solution prepared by adding the following reagents in the order indicated:

Distilled water	100.0 ml.
Sodium sulphite, anhyd.	10.0 Gm.
Hydroquinone	1-2 Gm.
Urea	20-30 Gm.

Agitate the slides gently for about the first 2 minutes.

8. Wash thoroughly in 4 or 5 changes of distilled water.

9. Run sections through graded alcohols to 80 per cent alcohol and examine under the microscope. If the staining is not completed repeat steps 5, 6, 7, 8 and 9 as often as necessary. The original solutions may be used in this restaining, but the time the sections are kept in the urea-silver nitrate bath (step 5) should be reduced to 10 to 15 minutes.

10. When staining has been completed, dehydrate through 95 per cent and absolute alcohol, clear in xylol and mount.

The sections may be coated with 0.5 per cent celloidin in both methods before staining and also before mordanting in the cupric nitrate solution to prevent loss of sections and precipitation.

RESULTS

With Method I the nerve processes are stained black in contrast to the much lighter staining of the surrounding nonnervous structures. By this method and with serial sections the continuity of the nerve fibers from the pulp deep into

Fig. 5.

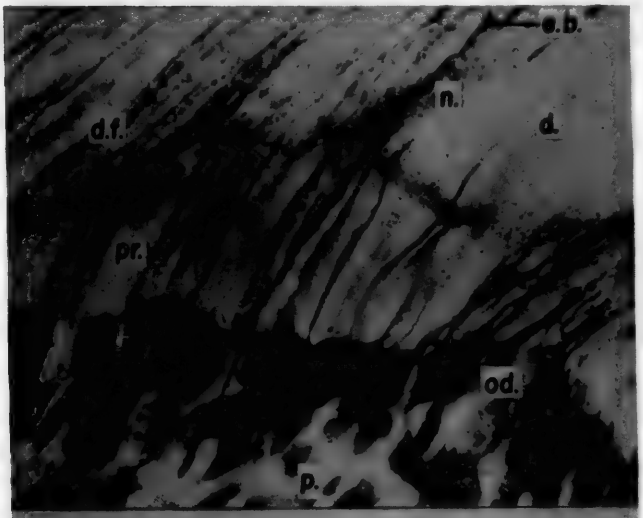

Fig. 6.

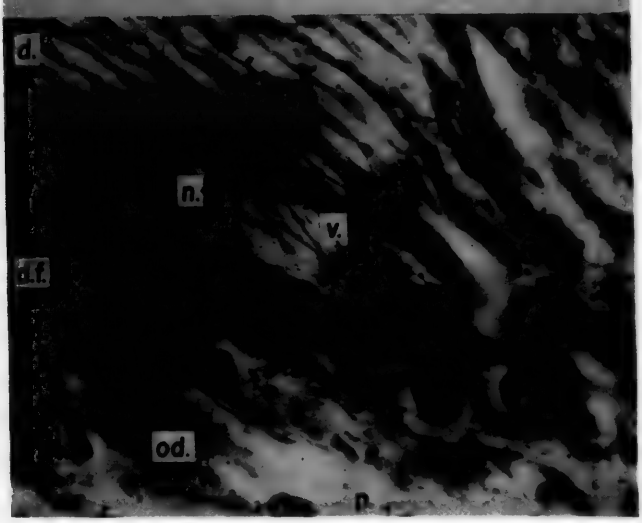

Fig. 5.—Longitudinal section through molar of rat. Method I. Section shows continuity of nerve fiber from Fig. 1 through Fig. 4 traced in order to bring the whole nerve fiber into focus. *n.*, nerve fiber; *pr.*, predentin; *d*, dentin; *p*, pulp; *e.b.*, end bulb; *od.*, odontoblast; *d.f.*, dentinal fiber.

Fig. 6.—Longitudinal section through molar of rat. Method I. Section shows nerve fibers extending from pulp into dentin. Note loop of nerve fiber. *n.*, nerve; *v.*, varicosity; *d.*, dentin; *p.*, pulp; *d.f.*, dentinal fiber; *od.*, odontoblast.

(For legends, see opposite page.)

the dentin may be traced (Figs. 1, 2, 3, and 4). Fig. 5 is a photograph of a longitudinal section through a rat molar showing the surrounding nonnervous structures with the continuity of this nerve fiber inked in to bring its whole course into view. The specific staining of an end bulb at the termination of this fiber suggests that this structure is a true nerve ending and not an "altered odontoblast entrapped in the dentinal matrix" as suggested by Bernick.[4] Fig. 6 shows nerve fibers extending from the pulp into the dentin and forming loops. In Figs. 1 through 6 there is clear differentiation of nerve fibers from nonnervous structures such as the odontoblasts, dentinal fibers and dentinal tubules. Figs. 7 and 8 represent sections taken through the long axis of a human premolar showing nerve fibers extending in the dentin and predentin zones. It is apparent from these figures that nerve fibers are not within the dentinal tubules as stated by Berkelbach[1] and Tojoda,[10] but between the tubules within the dentinal tissue itself. This observation is in agreement with the opinion of Bernick[4] that nerve fibers do not lie in the dentinal tubules. Along the nerve processes many varicosities may be seen as these processes continue to branch into fine filaments (Fig. 12) and loops (Fig. 6) which is in accordance with the findings of Tiegs[6] and Wasserman.[8]

With Method II the nerve fibers are stained a deep brown to black against an almost colorless background. Fig. 11 shows the looping of nerve fibers within the predentin of the crown of a human premolar. These loops pass from the odontoblastic layer through the predentin and back to the odontoblastic layer thus confirming the observations of Bernick[4] and of Wasserman.[8] These loops of fibers extend along the sides of the dentinal tubules and may pass transversely to the tubules and parallel with the odontoblastic layer (Figs. 10 and 11). It is to be noted that the same type of branching and looping of nerve fibers in dentinal tissue surrounding the dentinal tubules may be obtained with Method II (Figs. 9, 10 and 11) as in Method I (Figs. 7 and 8). Varicosities are prevalent on the nerve processes with Method II (Figs. 9 and 11).

It is evident that the use of both methods on human and rat teeth will give data unobtainable by other techniques. For example, the Ungewitter's method[18] gives a specific stain while with the technique of Romanes[17] there is sufficient staining of nonnervous tissue for accurate orientation. With Romanes's procedure dentinal fibers are stained and between the dentinal tubules are nerve fibers with varicosities forming a plexus. With the Ungewitter's technique the dentinal tubules are faintly stained, if at all, whereas the plexus remains, showing that it is truly a nervous plexus. Method I and Method II give excellent differentiation of the sections and the results are consistent.

Fig. 7.—Section through a human bicuspid. Method I. Portion of pulp (*p.*) with transverse section of darkly-stained dentinal fibers (*d.f.*) within dentinal tubules (*d.t.*). Nerve fibers (*n.*) branching in the dentinal tissue but not entering dentinal tubules.

Fig. 8.—Section through a human bicuspid. Method I. Nerve fibers (*n.*) in dentinal tissue among the transverse sections of stained dentinal fibers (*d.f.*). Dentinal tubules (*d.t.*) show faintly. Note varicosities (*v.*) on the nerve fiber and calcified globules of dentin (*gl.*).

Fig. 9.—Section through a human incisor. Method II. Section shows nerve fiber (*n.*) branching in predentin and dentin in apical region. Note varicosity (*v.*). Observe that nerve fibers do not lie within dentinal tubules (*d.t.*).

Fig. 10.—Section through a human bicuspid. Method II. Section shows that nerve fibers (*n.*) may pass obliquely across dentinal tubules (*d.t.*) and parallel to odontoblastic layer (*od.*).

Fig. 11.

Fig. 12.

Fig. 11.—Longitudinal section through a human bicuspid. Method II. Section shows looping of nerve fibers (*n.*) within the predentin (*pr.*) of crown of tooth. Note dentinal tubules (*d.t.*) and varicosity (*v.*) on nerve fiber (*n.*). *Od.* denotes region of odontoblastic layer.

Fig. 12.—Longitudinal section through molar of rat. Method I. Section shows branching of nerve fibers (*n.*) within the pulp (*p.*) and extending toward dentinal tissue. Note varicosities (*v.*) on nerve processes.

There have been several authors who purported to demonstrate an abundance of nerve fibers in the dentin. Many of these (Tojoda,[10] Münch,[11] and Berkelbach[1]) present figures showing what they call nerve fibers within the dentinal tubules. In the main these so-called nerve fibers are probably artifacts. It must be stressed that simply because a structure is stained with silver is no proof that the structure is a nerve. Silver may stain nonnervous structures rather selectively (Powers, Rasmussen and Clark[20]). Unless the structure has the morphology of a nerve fiber the possibility of nonspecific staining or of artifact must be considered. We have found that the use of the two methods given above aided interpretation, for with the first method there was sufficient impregnation of nonnervous structures to permit orientation, while with the second one the differentiation of nerve fibers from other tissue elements was certain. In specimens where the selectivity of the stain is poor and where precipitation artifacts are present it is possible that a nerve fiber might appear continuous with a Tomes's fiber. The structures said to be intratubular nerve fibers shown in Figs. 5, 6, 7, 8 and 10 of Tojoda[10] have little resemblance to nerve fibers and are probably examples of nonspecific staining. If all the structures shown by Tojoda in these figures were nerve fibers the dentin would have a greater number of nerve fibers per unit volume than any other structure outside the nervous system. It seems quite definite that the fibers entering the dentin may be quite near dentinal tubules (Fig. 1 and also Figs. 1 and 2 of Tojoda)[10] but in these it is easily seen that the nerve fibers shown are not intratubular. In our own material we have often seen fibers near tubules but have never seen intratubular nerve fibers. However, we would not wish to say that all dentinal nerves are extratubular (i.e., not present in dentinal tubules) but rather to stress that with our methods we have never seen intratubular nerve fibers. This investigation is being continued and it is hoped that it will be possible to present more definite evidence in the near future.

SUMMARY

The use of the Romanes[17] and the Ungewitter[18] silver nitrate techniques as necessarily modified for the demonstration of nerve fibers in the pulp and the dentin of adult human and matured rat teeth is discussed. In spite of numerous statements to the contrary it was possible to demonstrate that there are nerve fibers in the dentin and that in their course through this hard tissue the fibers present many varicosities and that they may terminate in so-called nerve endings. This report is tentative and further work is in process to determine the full extent of the dentinal plexus, the source of the nerve fibers, and the question of nerve endings.

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FURTHER EXPERIMENTS WITH INTACT TEETH PLACED IN VARIOUS BUFFER SOLUTIONS

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IN A recent experiment,[1] it was shown that the loss of weight of intact teeth placed in different buffer solutions at varying pH levels did not increase linearly with the time of exposure, but followed an S-shaped curve (Figs. 1a and 1b). The nature of the curve prompted further experiments using a greater number of teeth. It was felt that the initial lag period was so important clinically that a repetition along these lines, with the weighing of specimens at shorter time intervals, would be desirable. On the other hand, the flattening of the curve at the end of the experiment raised certain doubts as to its proper interpretation.

The problem of decalcification of tooth structure by acid buffers has been studied by various investigators. Several methods are available for measuring the degree of dissolution. It is probable that the best method, although it is more complicated and therefore is least used, would be microchemical quantitative determination of minerals in solution. Karshan and Rosebury[2] used a simpler approach, determining dissolved P at each time level. They found that, in lactate buffers, Ca values are about twice P values. With regard to the anions, Eriesson[3] has recently stated that "the molar ratio of 0.67 between phosphorus and calcium dissolved lies near the P:Ca ratio found for the enamel's total mass," and "in buffered slightly acid liquids, the phosphate dissolution is a convenient indicator."

The method generally adopted and one which was, for the most part, used by us is the gravimetric method. It is an indirect method of checking weight losses and is based on the theory that the acid effect is proportional to loss of weight. The same method was recently used by Scrivener, Myer, Moore, and Warner.[4]

EXPERIMENTAL PROCEDURES

Twenty intact teeth, freshly extracted or kept in stock, were cleaned and washed under running water, and then dried with blotting paper. The crowns of six of these teeth were severed from the roots and the dentin removed with burs. The weight losses of the hollow enamel caps, thus obtained, and of the severed roots were determined separately. Before placing them in the various buffer solutions they were weighed on an analytical balance. The pH values were checked with the aid of a Hellige pH meter, with the accuracy of 0.1 to 0.02.

In addition, powdered enamel and dentin were used in our experiment for comparison. They were prepared, according to the method of Manly and Hodge,[5] to pass a 200 mash screen. Their weight losses were determined in filtering glass crucibles (Fig. 2). Powdered enamel was dried in an oven at

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120° C. for a half an hour, cooled, weighed, and placed in a lactate buffer solution of pH 4.85, in the proportion of 50 c.c. to each 0.500 Gm. of powder. With this proportion no significant deviation from the original pH values were observable. It was then filtered at intervals of 24 hours, washed with water in the crucible under suction, dried in an oven, cooled, and weighed again. The experiments were carried out at room temperature.

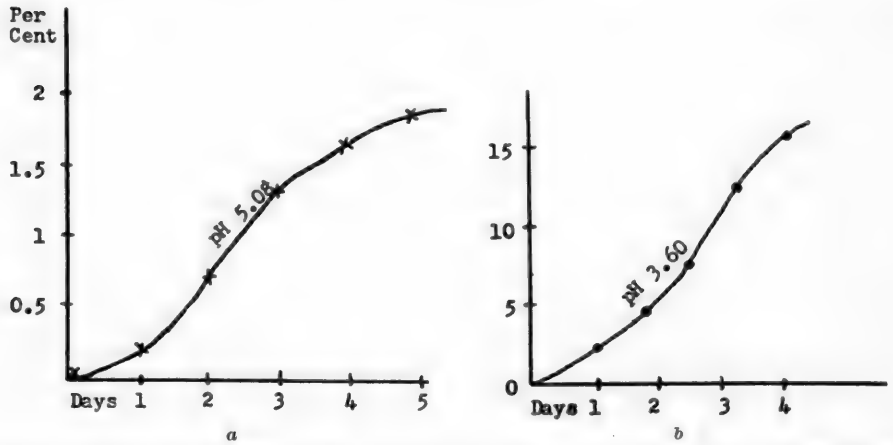

Fig. 1*a* and *b*.—Lactate buffer series at room temperature; percentage of loss of weight with relation to time. The dissolution curve has sigmoid form.

The weight losses were recorded and plotted in relation to time, each point representing three readings. The demineralization of powdered enamel and dentin appeared to follow a linear course (Fig. 3), which seems to be the rule for homogeneous structures. Fig. 3 shows that enamel is more resistant to acids than dentin. This conclusion from an experiment in vitro has corroborated the histologic evidence that caries stops before an hypercalcified layer, or at least, more time is required for acid diffusion to take place.

DISCUSSION

It has been erroneously postulated that the more mineralized enamel is more liable to decalcification than dentin. The fact is, that enamel contains larger apatite crystallites, is more compact and, therefore, less permeable to the acid solution. Evidently, it is not the quality of the mineral, which is the same, but the amount of organic channels and the different size of the apatite crystallites which they both contain. According to Armstrong,[6] those in dentin are smaller than those in enamel, and hence present a greater surface for adsorption.

With intact teeth, the process of dissolution does not proceed at a constant rate, but shows an initial lag period, the initial weight loss being insignificant. This was checked and recorded again in a new series of investigations (Figs. 4 and 5), in order to ascertain the minimum time for decalcification to begin, in

relation to a given pH. It has been said[7] that "the time that the acid must be in contact with the tooth in order to produce decalcification is not known." In vivo, it depends on the quality of the structure, the size of the apatite crystallites, and the width of the organic channels open for diffusion. On the other hand, the quality of the saliva undoubtedly plays an important role. According to McKay and Will,[8] some mouths cannot buffer cola solutions to a pH higher than 3.5 (marginal for erosion) after exposure of as short as half a minute.

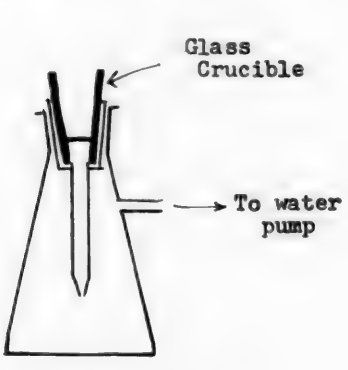

Fig. 2.

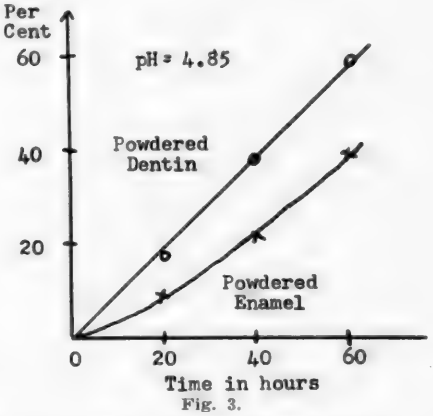

Fig. 3.

Fig. 2.—Filtering glass crucible attached to water pump; powdered enamel series.
Fig. 3.—Powdered enamel and dentin; percentage of loss of weight in relation to time. Demineralization of homogeneous structures follows a linear course.

Figs. 4 and 5 present the results of a study of the initial fragment of the S-curve, where the rate of decalcification was checked gravimetrically at 15 to 30 minute intervals. This experiment suggests that if a pH of 3.95 persists and continuously bathes exposed roots in the mouth for fifteen minutes, a loss of weight through acid erosion of 0.1 per cent may result. This, in itself, does not constitute actual caries, but it may become the initial link effective enough to make the tooth more susceptible to bacterial attack. If the acid attack is slow and discontinuous it may cause formation of demarcation bands, the well-established layer of hypercalcified enamel described by Mummery and others as the layer of transparent enamel. If other factors are absent the decalcification may be obliterated and the surface polished by efficient mastication and physiological tooth friction. Obliteration, however, is rarely possible with continued use of acid beverages. The actual pH changes in a susceptible mouth have been graphically illustrated by Hill and White.[9] They showed that in the short period of eight minutes the pH on the surface of the tongue, after a sucrose rinse, dropped to 4.6. This pH persisted for another eight minutes and then gradually returned to normal. The pH on the surface of the tongue may be indicative of the pH of saliva which bathes the tooth surface. Stephan[10] determined the drop of pH directly on the film covering the tooth surface.

The pH of the plaque, and how fast it is buffered, has not been finally established to the best of our knowledge. In addition, there is no real evidence to state that the saliva buffers would not be equally able to diffuse into the plaque.

The third phase of the S-shaped curve should be considered. The diminishing rate of dissolution apparently is in accordance with the clinical fact that carious enamel is more resistant to acids. What causes the gradual flattening of the curve? Is it an evidence of a sudden decrease in the rate of decalcification, or is it an artifact? It is well known that the gravimetric method is an indirect method. Among its shortcomings are: 1) a precipitate may form on the tooth, 2) there may be a reaction of the enamel and dentin proteins with the components of the buffer solution, 3) imbibition of the specimen increases with increased demineralization and resulting porosity, 4) the mineral content is depleted, or 5) possibly, it is the anions which determine the different rate of solubility; first the carbonates readily go into solution, then the phosphates, and finally the more resistant fluorides.

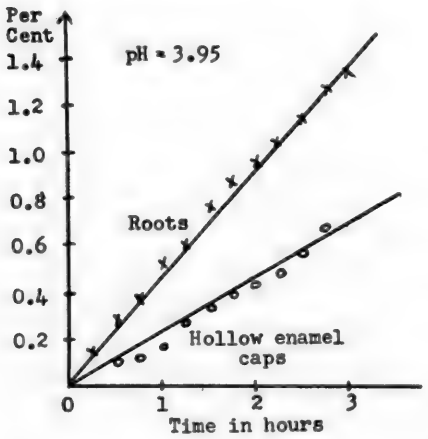

Fig. 4.

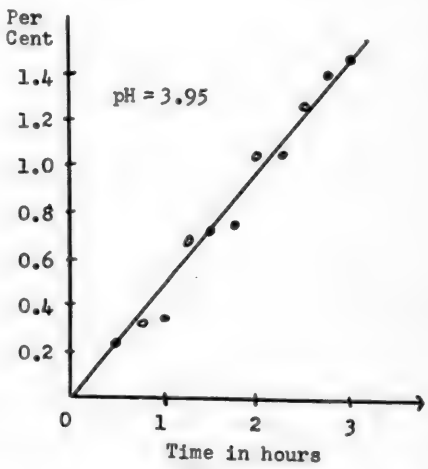

Fig. 5.

Figs. 4 and 5.—Lag period; lactate buffers at room temperature; percentage of loss of weight of hollow enamel caps and roots checked at fifteen to thirty-minute intervals.

A simple preliminary experiment made us realize the possible errors entailed in this method due to imbibition. If the experiment with acid buffers is extended long enough the specimen may even begin to increase in weight. If a tooth is immersed in a weak nitric acid solution and the weight checked every fifteen minutes the tooth will steadily lose weight for the first hour or so, becoming soft and pliable. Then the specimen begins to gain weight. In such a case the only plausible explanation seems to be that the tooth became porous to such an extent that the imbibed water could not evaporate under the conditions of the experiment, although visible precipitates were not uncommon in our experiments.

SUMMARY

A gravimetric method was used for determination of loss of weight, in relation to time, of intact tooth, or powered enamel and dentin, and of enamel caps and roots of human intact teeth. The loss of weight follows an S-shaped curve.

Enamel caps bathed in lactic buffers of pH 3.95 for 15 minutes at room temperature, lose about 0.1 per cent of their weights. At higher levels of pH and in the so-called "lag period," the initial decalcification is almost insignificant. If the acid action is continuous, or at a lower pH-level, the loss of weight gradually increases, the curve assuming an exponential character.

Dentin is less resistant to decalcification by acids on account of its greater permeability, its greater number of organic channels and the smaller size of its apatite crystallites.

The gravimetric method with air-dried specimens has limited use since increased imbibition of the specimen through continued demineralization may lead to false determinations.

Experiments were especially designed to study the dissolution rate at short time intervals, instead of days. Such study was considered desirable, as it more closely approaches the clinical occurrence of dissolution of tooth structure in the oral cavity.

The separation of enamel and dentin and the establishment of a different rate of their solubility was given special consideration, as it bears on our evaluation of the different conceptions of the mechanism of caries.

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EARLY INFLUENCE OF METHYLCHOLANTHRENE ON TRANSPLANTED TOOTH GERMS: A HISTOPATHOGENIC STUDY

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IN THE course of recent investigations it was found by this investigator[1] that the initial cellular attack on tooth germs of mice, homologously transplanted to the anterior eye chambers, was of such a nature that growth and differentiation of these transplants at this site were infrequent. However, at other sites in mice, in the eyes and brains of guinea pigs, and in the eyes of rabbits growth and differentiation of transplanted tooth germs were usually obtained. The behavior of certain tissues is altered when transplanted with carcinogens of a known molecular arrangement.[2] It is felt that the course of development of tooth germs when synchronously transplanted with a known carcinogen such as methylcholanthrene are sufficiently changed and of sufficient interest to warrant a report. It is the purpose of this paper to present these findings.

MATERIAL AND METHODS

The axillae of mice and brains of guinea pigs were selected as sites for transplantation because of the regular development of tooth germ transplants in these places. Tooth germs at the cap stage of development were transplanted with several crystals of methylcholanthrene to the brains of guinea pigs and the axillae of mice. Fourteen-day dba mice embryos and 20 day guinea pig embryos supplied the tooth germs. Tooth germs were aseptically removed from the jaws of embryos and placed in sterile covered Petri dishes where they were kept moist with sterile normal saline until used. The trocars employed for transplantation were specially designed and ranged in size from No. 16 to No. 20. The tissue was impregnated with several methylcholanthrene crystals after it was loaded in the trocar. Incisions for axillary transfers were made at a distance from the transplantation site to prevent the transplant from being lost. The technique of brain transfers has been previously described by Greene.[3]

Young adult dba mice, in groups of 50 which were evenly divided as to sex, received axillary transplants of dba tooth germs with methylcholanthrene. Five groups of mice received these axillary transplants of tooth germs with methylcholanthrene. With each group, about 20 additional young adult dba mice received transplants without methylcholanthrene and served as controls. In groups I and II, after the first 7 days, several mice bearing the transplant with methylcholanthrene were sacrificed every fifth day up to the fifty-seventh day and, at the same times, control mice were sacrificed. In the remaining groups of experiments with mice, at least 50 days elapsed before the animals were sacrificed and the transplants recovered.

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Guinea pig tooth germs were synchronously transplanted with several crystals of methylcholanthrene to the brains of guinea pigs. There were five groups of these animals receiving brain transfers with 12 to 16 animals comprising each group. With each group, 4 or 5 additional animals received brain transplants of tooth germs without methylcholanthrene and served as controls. The young adult guinea pigs used were chosen at random without regard to sex. These transfers were allowed to remain in place for at least 60 days before the animals were sacrificed and this study represents findings up to approximately 90 days.

Animals with brain transfers were sacrificed in chloroform gas chambers to minimize brain damage. Animals with axillary transfers were sacrificed by cervical dislocation. Recovered transplants were fixed in 10 per cent formalin solution and were decalcified in an ionic bone decalcifier before sectioning. Sections were cut at 5μ and stained with hemotoxylin and eosin.

RESULTS

Gross Findings.—Transplants made with methylcholanthrene usually attained a larger size than those made without this carcinogen. At times bits of tooth structure and spicules of bone were found in recovered transplants. In the axilla transplants with methylcholanthrene attained a size of from 2 to 5 mm. in diameter, sometimes consisting of several joined lobules of cyst-like structures. In the brains of guinea pigs, transplants made with methylcholanthrene frequently formed several joined hard nodules from 1 to 2 mm. in diameter. In mice, approximately 90 per cent of the transplants made with methylcholanthrene were recovered. There was material to section in 72 per cent of the guinea pigs that had received brain transfers with this chemical agent. The sex did not have any apparent effect on the transplants. However, in the presence of methylcholanthrene in lactating mice, there was greater breast engorgement and duct proliferation on the side having the transfer.

Microscopic.—Transplants of tooth germs made without methylcholanthrene formed mature tooth structures. Depending on their activity at the time the transplants were recovered, ameloblasts were seen in various stages of development and odontoblasts were usually seen in a normal position along the periphery of the pulp. Axillary transplants made without methylcholanthrene showed a progressive development of tooth structures; in Fig. 2 which represents 17 days development in the axilla, tooth development is at the appositional stage. Enamel and dentin matrices have been laid down, but have not yet been entirely calcified and both ameloblasts and odontoblasts are in a normal pattern.

There is striking difference in Fig. 3, which represents a transplant of a tooth germ made with methylcholanthrene crystals and carried for the same length of time as in Fig. 2. Instead of tooth structures, there is a large area of newly formed keratin which is surrounded by several layers of epithelial cells. The nuclei of these epithelial cells along the periphery of the keratin are hyperchromatic and pyknotic. A few of these cells, in the process of being keratinized, have retained their distorted nuclei. The intima of some of the blood vessels adjacent to the area described above are thickened. Groups of blood vessels in certain areas are heavily engorged with red blood cells.

The growth of a tooth germ in the axilla of a dba mouse for 32 days is represented in Fig. 4. There is dentin formation, and enamel crystals are seen in the cusp area. Ameloblasts in a partially keratinized condition lie against the crown surface of this tooth structure which is almost completely surrounded by bone. Infiltrations of small round cells are seen in several places. In this section, blood vessels are not engorged with red blood cells. Lack of keratin formation is significant.

Fig. 5 shows the recovered transplant of a tooth germ with methylcholanthrene after 32 days in the axilla. The presence of this chemical agent did not always prevent the formation of calcified tooth structures but, as in the present instance, it seemed to have its greatest effect on the epithelial tissues of the tooth germ. This section is interesting because it shows the relation of the outer epithelial layer of the enamel organ to dentin under induced degenerative conditions. Irregular keratin formation lies between the dentin and this outer epithelial layer which has become thickened. Along the inner portion of this layer, most of which is forming keratin, small whorls of cells are seen. Keratohyaline granules are seen in some of these cells, de-differentiating to form keratin. In places, the outer layer of this epithelium is columnar and some of these cells appear to be undergoing vacuolar degeneration. Nuclei of these cells vary in size and appear to have acquired aggressive characteristics because they seem to be infiltrating adjacent muscle tissue.

Fig. 6 is shown because it presents ameloblasts undergoing vacuolar degeneration. This section represents a part of a tooth germ transplanted to the axilla of a dba mouse with methylcholanthrene and recovered after 50 days. The effect of the chemical agent in this case was not as great as in other instances, but it shows how the cells of the stratum intermedium move away from the ameloblasts when the latter undergo degenerative changes. Thrombosed blood vessels are seen in the remains of the enamel pulp. The cells of the stratum intermedium have moved away from the ameloblasts in cords or chains and have obliterated the stellate reticulum of the enamel pulp.

The recovered transplant of a tooth germ after 57 days in the axilla with methylcholanthrene is illustrated in Fig. 7 and represents a frequent finding in these experiments. Usually in these instances there are 3 or 4 joined areas which are lined with epithelium. One or 2 of these areas may have wavy keratinized material and an occasional fragment of tooth structure. These latter areas represent the epithelial remains of transplanted tooth germs. Some of the epithelial cells bordering these areas have retained a semblance of their previous pallisaded

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KEY TO SYMBOLS

A—Ameloblasts
Art.—Artifact
B—Bone
BV—Blood Vessel
Br.—Brain
Cart.—Cartilage
CT—Connective Tissue
Cy. D.—Cystic Degeneration
Cy.—Cyst
Cyt.—Cytoplasm extruded
D—Dentin
D. ep.—Degenerated Epithelium
E—Enamel
Ep.—Epithelium
Ep. C.—Epithelial Cells
KA—Keratinizing Ameloblasts
K—Keratin
MI—Mitosis
M—Muscle
O—Odontoblasts
Od—Osteodentin
O.E.Ep.—Outer Enamel Epithelium
Tg—Tooth germ
Th.BV—Thrombosed Blood Vessel
SI—Stratum Intermedium
Vac—Vacuolar Degeneration

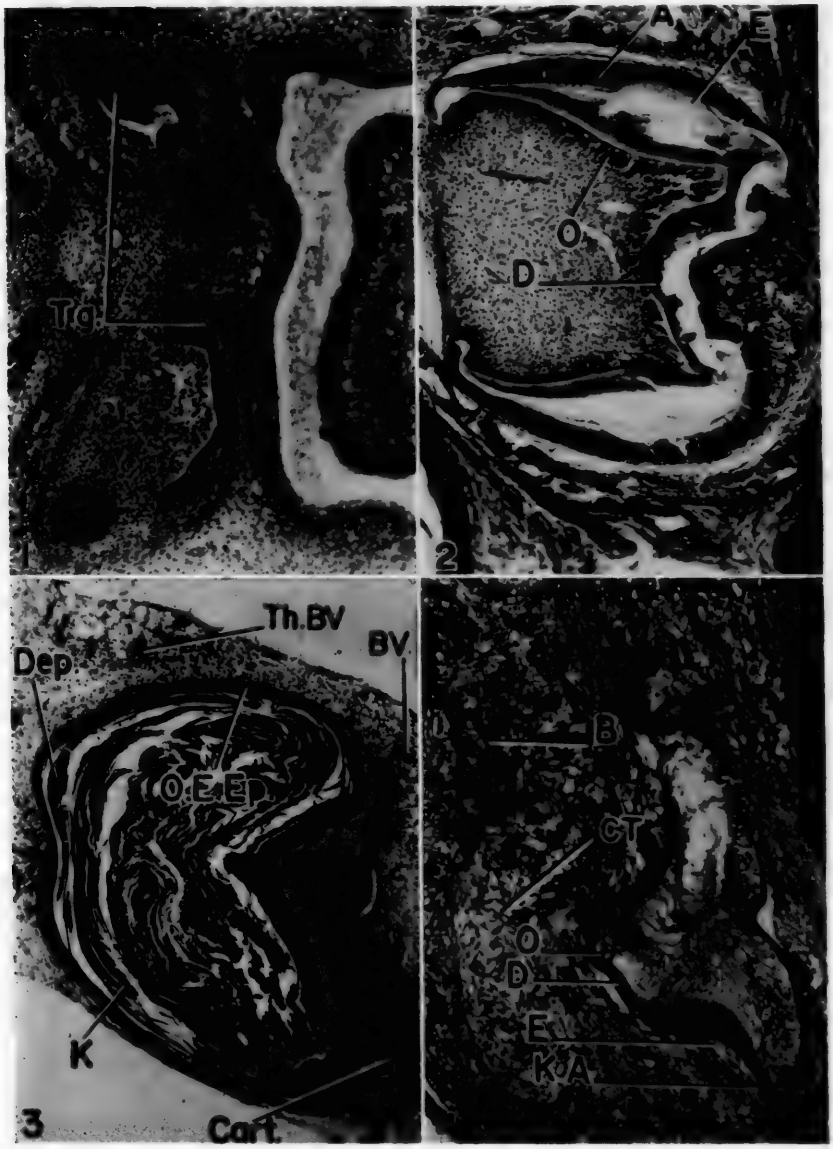

Fig. 1.—Cross section of head of 14 day dba embryo through molar tooth germs. Original magnification ×140.

Fig. 2.—Section of molar tooth after 17 days in axilla of male dba. Anlage obtained from 14 day dba embryo. Original magnification ×100.

Fig. 3.—Section of molar tooth after 17 days in axilla of male dba with methylcholanthrene. Anlage from 14 day embryo. Original magnification ×95.

Fig. 4.—Section of molar tooth after 32 days in axilla of female dba. Anlage from 14 day embryo. Original magnification ×175.

(*For key to symbols, see opposite page.*)

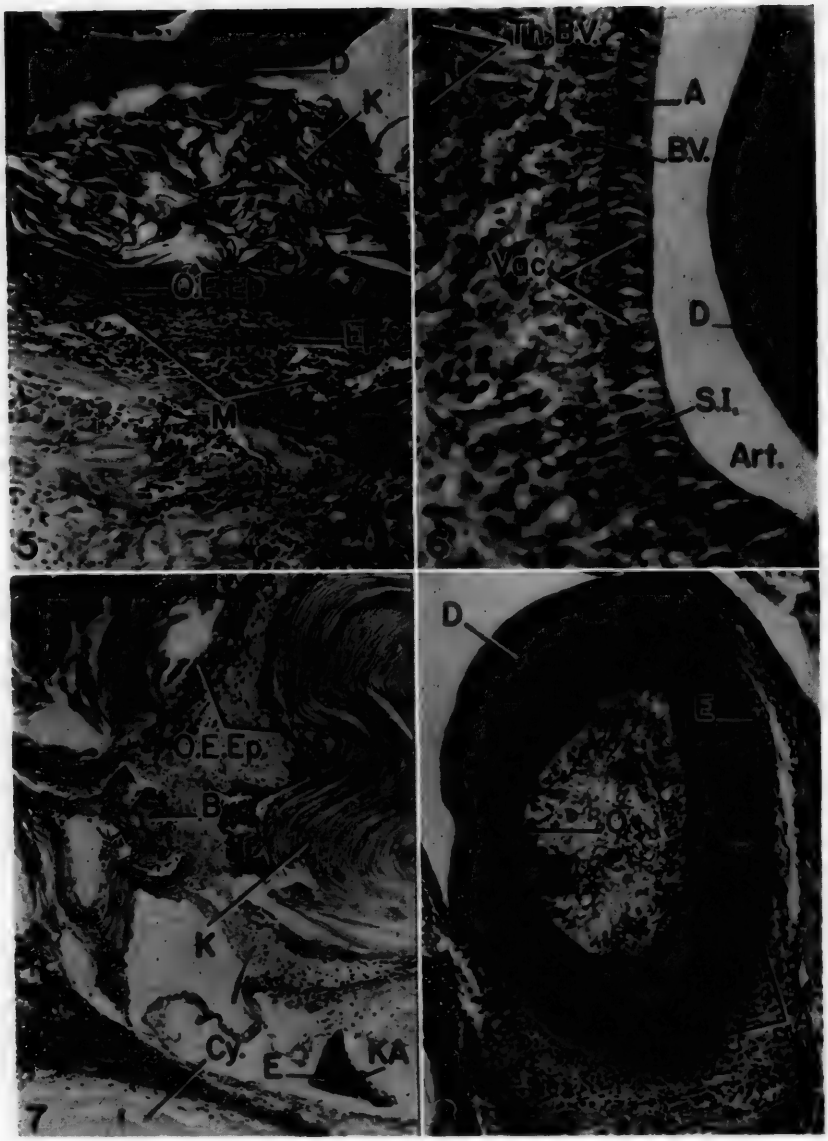

Fig. 5.—Cross section of molar tooth from 14 day embryo after 32 days with methylcholanthrene in the axilla of a female dba mouse. Original magnification ×240.

Fig. 6.—Ameloblasts from anlage of 14 day embryo after 50 days with methylcholanthrene in axilla of a dba female. Original magnification ×525.

Fig. 7.—Recovered transplant of dba tooth germ from 14 day embryo after 57 days with methylcholanthrene in a female dba. Original magnification ×165.

Fig. 8.—Cross section of a tooth in axilla of a male dba after 57 days. Anlage from 14 day embryo. Original magnification ×190.

(*For key to symbols, see page* 400.)

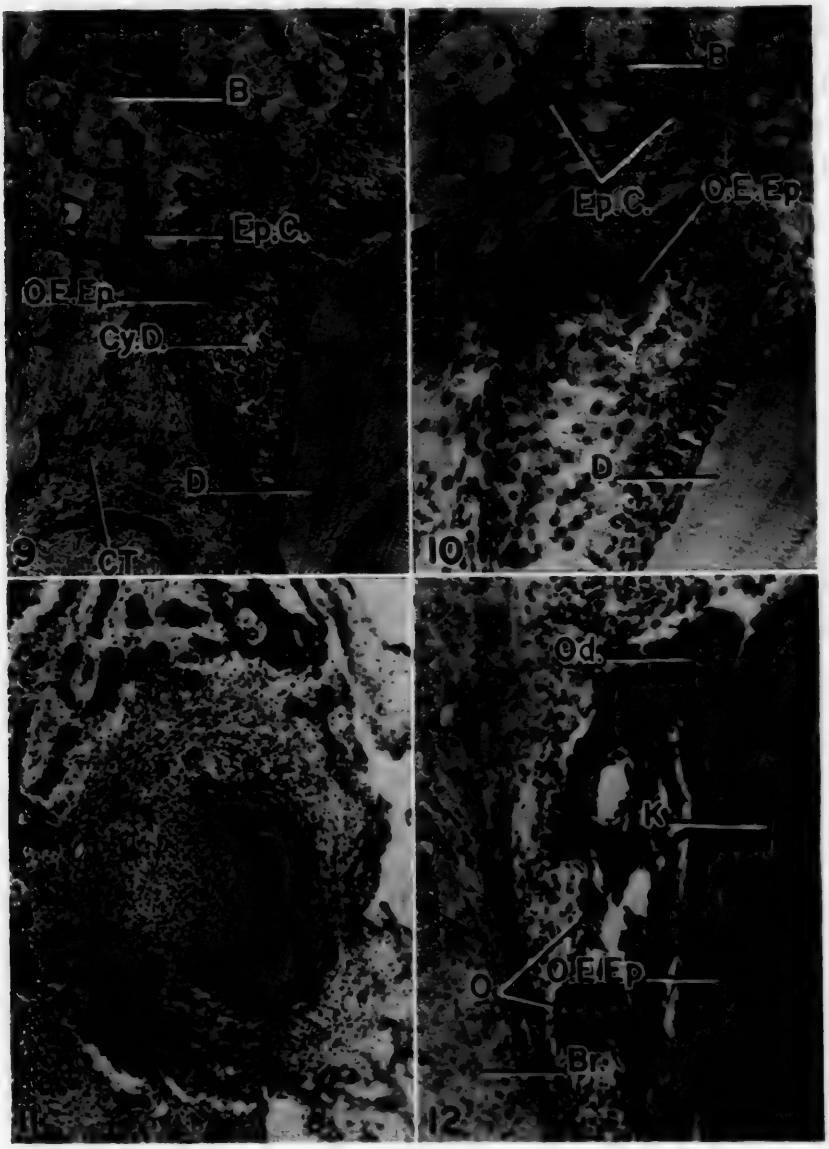

Fig. 9.—Section of recovered transplant of tooth germ from 14 day embryo after 65 days with methylcholanthrene in axilla of dba male. Original magnification ×220.

Fig. 10.—Higher power of Fig. 9. Original magnification ×400.

Fig. 11.—Tooth germ from 20 day guinea pig embryo. Original magnification ×130.

Fig. 12.—Odontoblasts and osteodentin after 65 days in female guinea pig's brain with methylcholanthrene. Original magnification ×190.

(*For key to symbols, see page* 400.)

TABLE I

	HOSTS	STRAIN	SEX	MATERIAL	DOSE	SITE	DURATION	SURVIVAL	REMARKS
I	50 mice	dba	About evenly divided	14 day dba embryos supplied tooth germs for all mice experiments	Several crystals of 20 methyl cholanthrene was placed on the tissue just before transplantation	Axilla	After *i* days animals sacrificed every 5 days to the 57th day	Generally all	Transplants recovered in 90 per cent of the cases and showed general retardation of tooth development
II	50 mice	dba	About evenly divided	14 day dba embryos supplied tooth germs for all mice experiments	Several crystals of 20 methyl cholanthrene was placed on the tissue just before transplantation	Axilla	After 7 days animals sacrificed every 5 days to the 57th day	Generally all	Controls for groups I and II showed tooth development
III, IV, V	150 mice divided into 3 groups of 50	dba	About evenly divided	14 day dba embryos supplied tooth germs for all mice experiments	Several crystals of 20 methyl cholanthrene was placed on the tissue just before transplantation	Axilla	Mice were sacrificed from 50-80 days	Generally all	Small tumors in most animals. Sometimes bits of calcified tooth material were found. Lactating mice sometimes had breast engorgement with proliferation of ducts. Controls showed tooth development
VI, VII, VIII, IX, X	72 guinea pigs in groups of 12-16 (total of 5 groups)	Open stock	About evenly divided	Tooth germs obtained from 20 day guinea pig embryos	Several crystals of 20 methyl cholanthrene was placed on the tissue just before transplantation	Brain	Guinea pigs were sacrificed from 60-90 days	7 lost to death and infection	In 52 of the survivors material was recovered which was mostly of a keratinous nature. Sometimes bits of tooth structure were found. Controls show tooth development

arrangement. The nuclei of these cells vary in size and are hyperchromatic and pyknotic. Ameloblasts at the end of their life cycle and undergoing keratinization appear to be lying against a fragment of enamel.

The 57 day growth of a tooth germ in the axilla of a dba mouse is represented in Fig. 8. Dentin and enamel are present.

The section shown in Fig. 9 represents an axillary transplant of a tooth germ made with methylcholanthrene and recovered after 65 days. Examination of serial sections of this recovered transplant revealed dentin, but no enamel formation. Adjacent to this dentin formation are clumps and cords of epithelial cells. Several of the larger areas appear to be undergoing central degeneration or necrosis. Nearby bone appears infiltrated by some of these epithelial cells, thus giving a picture similar to that seen in Fig. 5 and found in other instances in this study. The cells of the outer enamel epithelium of the enamel organ, in the presence of methylcholanthrene seem to assume aggressive characteristics when they become disassociated with tooth development. Fig. 10 represents a higher magnification of an island of epithelial cells seen in the previous illustration.

Fig. 11 represents a control for guinea pigs being a tooth germ from a 20 day embryo. Tooth germs from other embryos of this litter were transplanted and are represented in some of the succeeding illustrations.

Fig. 12 shows the recovered growth of a tooth germ grown in the brain of a guinea pig with methylcholanthrene for 65 days. Epithelial remains of the tooth germ are represented by the large area of keratin bordered by several layers of epithelial cells. An area of osteodentin is seen in this picture with odontoblasts arranged in a regular fashion along one side. Because of degenerative changes these odontoblasts have assumed an appearance like osteoblasts. The Tomes' fibers appear broken off or missing in most of these cells. Other odontoblasts are scattered in the remains of the pulpal area. The cytoplasm of these cells, especially those bordering the osteodentin formation, has a granular appearance and these cell walls lack definite boundaries. Several included cells are in the osteodentin. A higher magnification of an area of these odontoblasts is seen in Fig. 13.

In Fig. 14 the transformation of ameloblasts into keratin is seen. This section represents the recovered transplant of a guinea pig tooth germ from a 20 day embryo which was transplanted to the brain of a guinea pig with several crystals of methylcholanthrene. The transplant was recovered upon sacrificing the host after 65 days. Through the center of this picture there are many ameloblasts in various stages of dyskeratosis. As these cells become keratinized, their cell walls or cuticular borders thicken as their cytoplasm decreases. The nuclei of these cells usually are extruded, but many are retained along the periphery of the keratinized area. During this process these cells seem to be very elastic for they elongate and adhere to adjacent cells thus making long strands of keratin. As the amount of cytoplasm becomes smaller, these cells change in staining reaction from basophilic to eosinophilic. The nuclei also take a heavy

basophilic stain at first, but later this becomes very faint. With completion of keratinization the staining reaction of the cuticular remains is much lighter. Fig. 15 shows a higher magnification of Fig. 14.

Fig. 16 shows the recovered transplant of a tooth germ after 73 days with methylcholanthrene in the brain of a guinea pig. In the center of this picture there are several discrete nodules of keratin. This section presents various combinations of calcified and uncalcified tooth structures with keratin, epithelial, and mesenchymal cells. In the center of this picture there is a large area of homogeneous hemotoxylin stained material which appears to be the ground substance of very young enamel. This is further emphasized because some of this area is cut to show cross sections of Tome's processes. Along the periphery of this area, numbers of ameloblasts are in the process of forming keratin.
Long thin particles of calcified tooth structure are seen in the upper part of this picture. Mitotic figures are seen among nests of epithelial cells.

Fig. 17 represents the recovered transplant of a tooth germ after 90 days with methylcholanthrene in the brain of a guinea pig. Remaining cells of the outer epithelial layer of the enamel organ are participating in the formation of keratin. Some of these cells are still arranged in a columnar fashion while others appear to be infiltrating adjacent brain tissue.

Fig. 18 represents a transplant of a tooth germ to the brain of a guinea pig without methylcholanthrene after 80 days.

DISCUSSION

Growth and differentiation of tooth germs transplanted with methyl cholanthrene appeared considerably retarded and altered. The toxic effect of this chemical agent appeared to be manifested in varying degrees during the initial weeks, but, over longer periods, there was no great variance between recovered transplants. Early variations may have been due to differences in contact between the methylcholanthrene crystals and the transplants. According to Page,[4] at times when methylcholanthrene was applied to the skin of mice marked effects were observed as early as 7 days with increase in nuclear size and cell layers. In the present experiments, similar effects on the outer enamel epithelium were observed in 12 days, by 17 days in many cases, and the outer enamel epithelium had de-differentiated into layers 6 to 8 cells deep. Page[4] stated that this chemical agent increased the amount of cornification of the skin but did not induce an inflammatory reaction. Recently, Firminger and Stewart[5] reported that crystalline methylcholanthrene caused an acute inflammatory reaction at the injection sites in the submucosa of the forestomachs of mice. The present experiments support the latter investigators observations, particularly on the point of inflammation; for in axillary transplants in mice, neutrofils, cell debris, and necrotic material were identified in cystlike areas where phagocytosis of fragments of methylcholanthrene was taking place. Later, the presence of plasma cells and round cells, in some of these cases, indicated a chronic inflammatory reaction.

The initial effects resulting from the presence of methylcholanthrene appeared to be on the blood supply to the transplant. Vessels supplying the

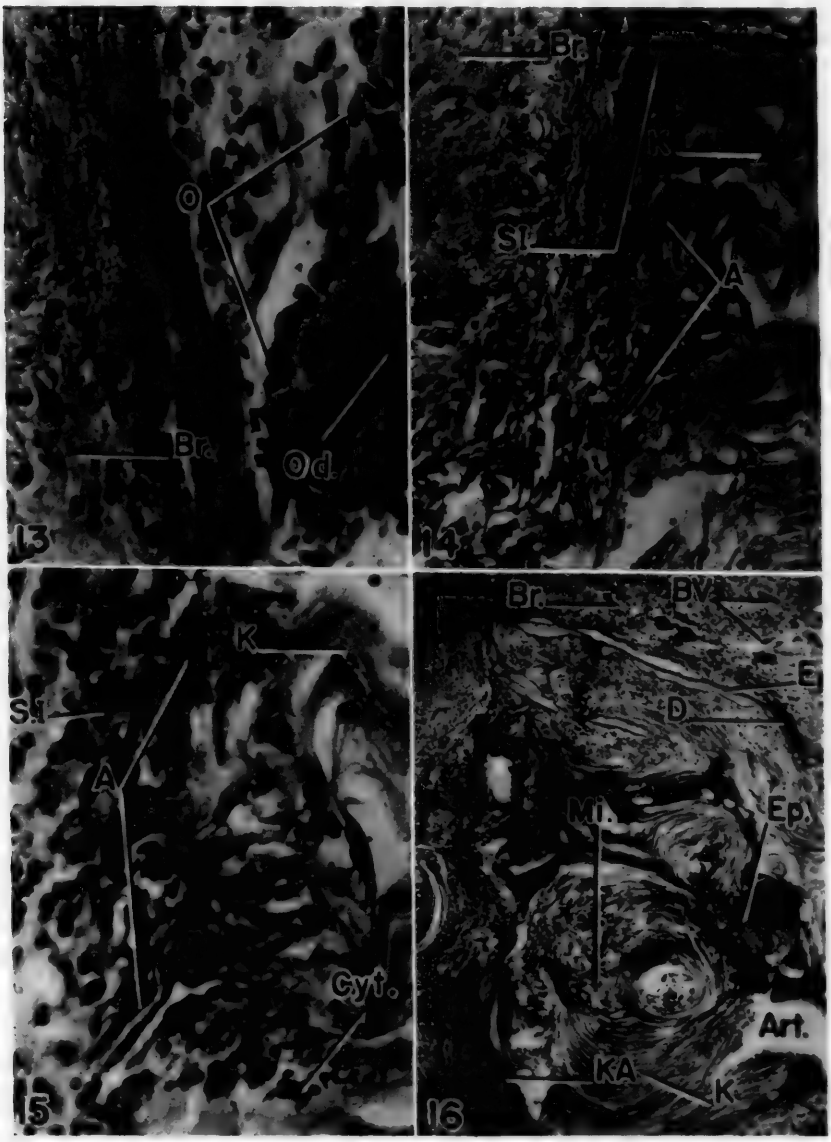

Fig. 13.—Higher power of Fig. 12. Original magnification ×630.

Fig. 14.—Ameloblasts in brain of male guinea pig after 65 days with methylcholanthrene. Anlage from 20 day embryo. Original magnification ×300.

Fig. 15.—Higher power of Fig. 14. Original magnification ×630.

Fig. 16.—Transplant in brain of male guinea pig after 73 days with methylcholanthrene. Anlage from 20 day embryo. Original magnification ×80.

transplant would often appear heavily engorged with blood and the intima of some were thickened so that the flow of blood must have been retarded. In addition, the blood supply was further restricted by the plugging or thrombosing of the lumina of other small vessels. It is probable, that these conditions either prevented development of tooth structures entirely or kept them at a minimum, for at no time was the complete development of tooth germs observed in the presence of methylcholanthrene. Presumably, the brain's higher vascularity in comparison to the axilla's may have been responsible for a lack of enamel formation at the latter site. Wasserman[6] observed that amelogenesis greatly increases the demand for fluids by the enamel organ. Previously Addison and Appleton[7] reported that the number of blood vessels increase and penetrate through the stellate reticulum of the enamel pulp to the cells of the stratum intermedium. Thus the restricting of the blood supply would have its greatest effect on the cells of the inner enamel epithelium.

The consistent presence of keratin or keratinous material in these experiments has an important significance. Chase[8] made observations on the origin and structure of Nasmyth's membrane, finding that cells of both the inner and outer epithelial layers participated in forming this cuticle. The present experiments confirm the previous investigators findings and also clarifies some additional points on keratinization. Orban[9] stated that when enamel has been completely developed, ameloblasts can no longer be distinguished from the cells of the stratum intermedium for at this time they become squamous in appearance and form a thin continuous layer over the entire enamel surface. The present study brought out that these cells need not assume a squamous appearance in order to form this keratinous substance. At certain stages during their life cycle, ameloblasts have an inherent dual functional capacity in that they may form enamel and subsequently form a keratin-like material from the cell remnants. Whether enamel is formed or not the cuticular parts of the cell will always form keratin. Since the ground substance of enamel is formed in the cytoplasm of the cell and keratin comes from the cuticular walls, a rapid loss of cytoplasm due to the indirect effects of methylcholanthrene may not allow the cell to assume a squamous appearance. During keratinization Chase[8] stated that the cuticular walls of the ameloblasts thickened as their cytoplasm decreased and their shrunken nuclei stained more deeply. In these experiments, keratinization appeared to take place so rapidly that nuclei were often retained in the keratin and gave the appearance of "ghost nuclei," because of their faint staining reaction. In the present study the cytoplasm of ameloblasts appeared basophilic while the limiting membranes of the cells were eosinophilic in staining reaction. Therefore, as the cytoplasm was lost, a transition in staining reaction from basophilic to eosinophilic seemed to take place. These observations of staining reactions of ameloblasts in the present study are not in agreement with Chase's[10] later findings, who stated that the interprismatic substance was basophilic in reaction and the substance of the prisms was eosinophilic. Some of Chase's[8] work on the transition of ameloblasts from columnar cells to flattened squamous cells was corroborated by Anderson.[11] In addition to Orban's[9] and

the above investigators findings, the present study brought out that the keratinous material formed from Tome's processes of individual ameloblasts appeared to have both an elastic and adhesive quality. As a result, strands of keratin became greatly elongated and adhered to each other in multilayered curved or wavy formations. The elasticity of keratin is explained by Bell, Davidson, and Scarborough.[12] They state that the main characteristic of keratin is elasticity, due to the ability of molecular chains to fold and unfold; thus, this fibrous scleroprotein is ideal for structural purposes. Since, as Orban[9] states, discussion is still active concerning the interrod substance cementing the enamel prisms together, the findings in the present study have added significance for they demonstrate the elasticity and adhesiveness of the cuticular walls of amelo-

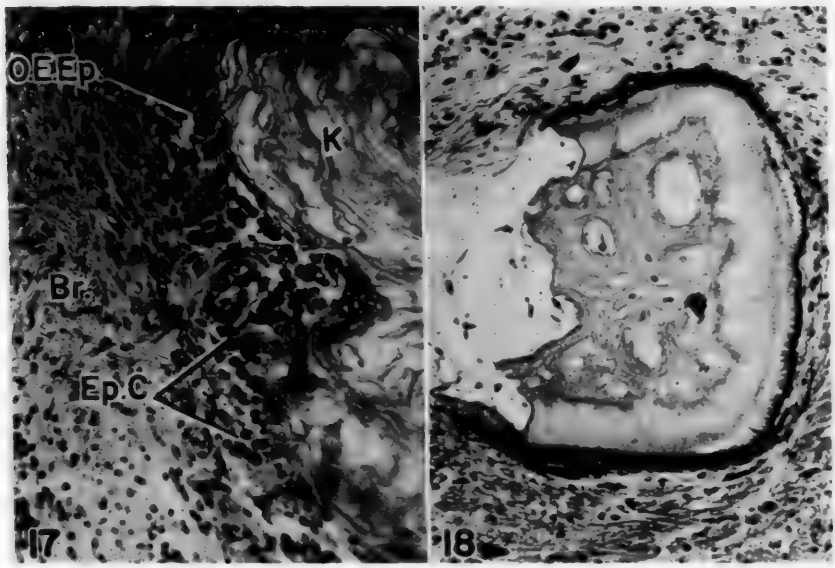

Fig. 17.—Transplant of tooth germ after 73 days with methylcholanthrene in the brain of a female guinea pig. Original magnification ×315.

Fig. 18.—Transplant of tooth germ from 20 day embryo after 80 days in the brain of a female guinea pig. Original magnification ×260.

blasts. It is therefore possible, since each enamel rod proceeds outward from the dentino-enamel junction to the tooth surface, that this cuticle helps cement the rods together as it stretches for the length of each enamel prism. Furthermore, it may also explain Wasserman's[6] findings in that he stated Tome's processes spread out in a fan shape crossing and intermingling with neighboring processes to form columns of the prismatic substance of enamel. Thus, it is possible that due to excessive stretching the cuticular walls might rupture near the dentino-enamel junction and thus the ground substance of the enamel rods might appear to overlap or fuse with one another.

Wolbach and Howe[13] and Boyle[14] reported on the effect of Vitamin A deficiency and ascorbic acid deficiency in rats and guinea pigs. They found that

these dietary deficiencies produced a loss of polarity and atrophy of odontoblasts. As a result, modified dentin was formed with atrophic included cells in the matrix. In previous experiments dealing with the transplantation of tooth germs[1] it was found that under certain conditions, changes in the odontoblasts similar to those described above took place. In the present study, dentin formed more frequently than enamel and it is reasonable to conclude that methylcholanthrene had a more toxic effect on the epithelial components of the tooth germ than on its mesenchymal counterpart.

According to Cramer and Stowell[15] the carcinogenic property of a hydrocarbon depends on its ability to inflict tissue injury of a specific kind leading to the formation of a substance in the skin that induces epithelial proliferation. The rate of epithelial proliferation, however, in the presence of a carcinogen is not increased above that occasioned by other forms of injury, according to Howes.[16] In addition to the above observations, Firminger and Stewart[5] noted focal proliferations of basal cells 17 days following injection. In view of the above observations the activity of the peripheral cells of the outer enamel epithelium may be explained. Furthermore, it has been previously shown that both the cells of the stratum intermedium and the outer enamel epithelium show irregular proliferation when ameloblasts degenerate.[17] These peripheral epithelial cells frequently appeared to assume aggressive characteristics in that they infiltrated adjacent structures. Thus in the axilla, muscle, and near-by bone (that accompanied the transplant) was infiltrated and in intracerebral transplants these cells began to penetrate adjacent brain tissue. The failure of ameloblasts to differentiate in the presence of methylcholanthrene and withdrawal of cells from the stratum intermedium has an important bearing on the histopathology of enamel hypoplasia. It appears that some of the cells associated with amelogenesis, the cells of the stratum intermedium, can alter their potentiality or fate when the development of ameloblasts is inhibited. It is therefore possible the location of these cells, or the site where they are to function, has an important bearing on their ultimate disposition.

SUMMARY

1. Growth and differentiation of tooth germs, when transplanted with methylcholanthrene are retarded.

2. The presence of methylcholanthrene seemed to have a greater effect on the epithelial cells of the tooth germ than on the mesenchymal cells.

3. Keratin formation is generally present arising from both the inner (ameloblastic) and outer enamel epithelium.

4. Under these conditions peripheral cells of the outer enamel epithelium show aggressive characteristics and may invade adjacent structures.

5. The cells of the stratum intermedium move away from the basal side of the ameloblasts in chains or cords.

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THE EFFECT OF ALTERATIONS OF THE FACIAL MUSCULATURE ON THE FACIAL SKELETON AND DENTAL ARCH FORM OF THE RAT

A Preliminary Study

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ORTHODONTISTS, on the basis of clinical observations, have long felt that the facial musculature plays an important role in the establishment of alveolar arch form and dental occlusion. The influence of the buccal and labial muscles in determining the direction of alveolar growth and the eruption of the teeth has been discussed by Brodie,[1] and the clinical significance of the lips and cheeks has been noted by him and others.[2] For many years myofunctional therapy has been used in orthodontic treatment as a means of altering unfavorable patterns of muscle function.[3]

There has been little experimental evidence reported concerning the effect of the facial musculature upon the bony architecture of the face and alveolar arches. Washburn[4] produced asymmetries in the nasal and maxillary bones of rats and rabbits by unilateral sectioning of the seventh nerve; this procedure, of course, was not designed to study the effect of individual muscles upon the bony skeleton, but rather the effects of total unilateral paralysis of the facial muscles.

The intertwining and decussation of the oral musculature interfere with evaluation of these structures as individual units. In this preliminary study we have endeavored to overcome this obstacle by altering the function of various components of the oral musculature in order to evaluate effects of such procedures on the developing facial skeleton.

MATERIALS AND METHODS

Thirty Sherman strain rats were used in this study; these were divided into six groups, a total of 18 serving as experimental animals; the remaining littermates served as controls. Portions of the lips and cheeks were dissected away under ether anesthesia, when the animals were about 30 days old and weighed approximately 40 grams. Recovery was uneventful. Six different types of operations were performed: (A) The buccinator muscle was removed unilaterally from the corner of the lip to the anterior margin of the masseter muscle; (B) the buccinator was removed bilaterally; (C) the superior portion of the orbicularis oris muscle was removed (the corners of the lips were not included); (D) the inferior portion of the orbicularis oris was removed, the corners of the lip remaining intact; (E) the superior and inferior portions of the orbicularis oris were removed unilaterally; (F) the entire orbicularis oris was removed.

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Operated and control animals were sacrificed at about the age of 4½ months, subjected to gross examination, and the skulls macerated, cleared, and dried. A series of measurments, to the nearest 0.1 mm. was obtained from the dried skulls with a finely pointed divider and Boli Gauge. Various dimensions were recorded for both the operated and control animals: 1) *superior cranial length*—from the anterior margin of the nasal bones to the posterior lambdoidal suture in the midline; 2) *superior cranial width*—the greatest distance between the lateral surfaces of the parietal bones; 3) *length of the skull base*—from the most anterior point on the premaxillary suture to the most posterior and inferior point on the occipital bone in the midline; 4) *palate length*—from the most anterior point on the premaxillary suture to the most posterior point on the palatine suture; 5) *palate width*—the distance between the posterior occlusal fossae of the right and left second maxillary molar teeth; 6) *zygomatic width*—the greatest distance between the lateral surfaces of the zygomatic bones; 7) *length and width of the nasal bones*—in their greatest length and width; 8) *length of the maxillary diastema*—from the most anterior point on the premaxillary suture to the most prominent point on the anterior surface of the first maxillary molar tooth; 9) *length of the mandibular diastema*—from the most prominent point on the first mandibular molar tooth to a point on the lingual surface of the alveolar process at the level of *infradentale;* 10) *mandibular length*—from the most posterior point on the condylar head to a point on the lingual surface of the alveolar process at the level of *infradentale.*

OBSERVATIONS

Gross examination of the operated animals prior to maceration disclosed no apparent changes. Measurements were made upon the cleared, dried skulls, and the data evaluated. The mean values and their standard deviations were calculated for all of the measurements obtained from the twelve control animals. Mean values alone were used to represent the measurements of each group of three operated animals. When the average measurement of a dimension for a group of experimental animals differed from the mean value of that measurement for the control group by five times the standard deviation the change was considered significant, allowing for less than a 0.1 of 1 per cent probability that such change could have occurred by chance variation alone. It was felt that the small sample of operated animals in any one group precluded acceptance of findings which differed by less than five times the standard deviation as an indication that a significant change had occurred.

Analysis of our data reveals no appreciable difference between any of the operated groups and the control animals in measurements of the superior cranial length, superior cranial width, length of the skull base, zygomatic width, palate length, palate width, and mandibular length. Significant changes, however, were noted in the premaxillary area and in the nasal bones of animals in experimental Group F, in which the entire orbicularis oris muscle had been removed. The three animals in this group presented evidence of a significant reduction in the dimensions of the nasal bones. The average nasal width was 5.6 ± 0.1 mm. in the control animals; the average value for this dimension for Group F

was 4.6 mm. In the control group the mean nasal bone length was 17.8 ± 0.4 mm.; the average for Group F was 15.8 mm. In both cases the changes noted differ by at least five times the standard deviation. The average length of the nasal bones in the other five operated categories closely approximated the mean value of the control group. In Groups D and E the average width of the nasal bones differed from the control value by about three times the standard deviation. Smaller differences were noted for Groups A, B, and C. These findings are summarized in Fig. 1.

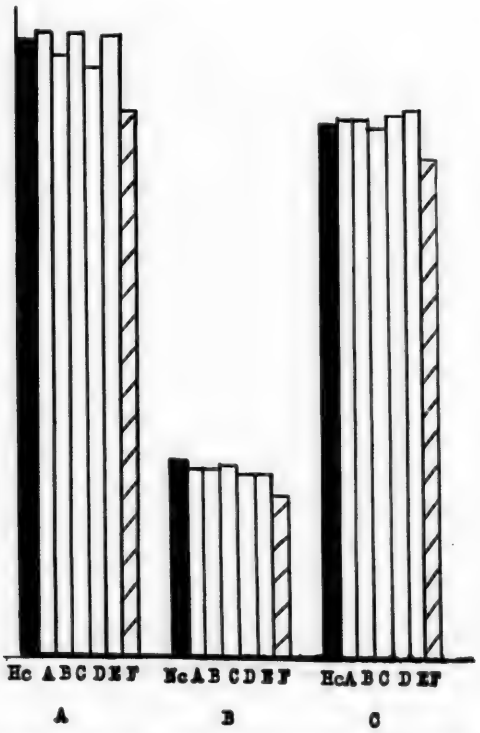

Fig. 1.—Histogram representing average measurements of similiar areas of the skulls in experimental and control animals. The solid columns represent the normal control group (Hc); the diagonally crossed columns represent significant findings; the letters from A-F represent the groups of experimental animals. *A*, nasal bone length; *B*, nasal bone width; *C*, length of the maxillary diastema.

A reduction in the length of the maxillary diastema was also found in Group F animals. In the control group the mean value of this dimension was 15.6 ± 0.2 mm.; the average for the three animals in Group F was 14.5 mm., a significantly different value. Average measurements of this dimension in the other five operated groups agreed closely with the mean value of the control group.

A reduction in the average length of the mandibular diastema was also noted in Group F animals, as compared with the controls, but the change was of the order of four times the standard deviation for that measurement (Controls, 7.0 ± 0.1 mm.; Group F, 6.6 mm.). This change may be regarded of

questionable significance. There were no appreciable differences between any of the other operated groups and the control animals in measurements of this dimension.

DISCUSSION

Of the various operations performed in this experimental study, the complete removal of the orbicularis oris muscle in one-month-old rats resulted in a reduction in the snout area, the only skeletal alterations of significance in the matured animals. This operation (Type F) affected several muscles associated with the orbicularis oris; the M. levator labii superioris, M. zygomaticus, M. buccinator and M. platysma. All are superficial muscles which intertwine with orbicularis oris, itself a poorly defined muscle in the rat.[5]

The changes noted were confined primarily to the bones of the nasal and premaxillary areas, while a somewhat less important change occurred in the area of the mandibular diastema. The findings indicate that the levator labii superioris and buccinator are the muscles most affected by the Type F operation, the platysma less so, and the zygomaticus very little, if at all.

Although alteration of the function of these delicate muscles was sufficient to produce significant changes in the dimensions of the alveolar arches and nasal bones as compared with littermate controls, no comparable effect on other skeletal dimensions studied could be ascertained. Our findings suggest that the nasal and premaxillary areas of the rat's facial skeleton are particularly responsive to changes in the function of the overlying facial musculature during the early stages of postnatal growth.

SUMMARY

1. Portions of the lips and cheeks were removed in a number of 1-month-old rats.

2. A series of measurements of various skeletal dimensions was made on the dried skulls following sacrifice of the experimental and control groups at about four and one-half months of age.

3. Those animals in which the entire orbicularis oris muscle had been removed showed evidence of reduction in the dimensions of the nasal bones and premaxillary area as compared with the normal control group.

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A RAPID RADIOAUTOGRAPHIC TECHNIQUE FOR COMBINED CALCIFIED AND SOFT TISSUES

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ALTHOUGH the first radioautographs were made almost 60 years ago[16] the use of this technique has not kept pace with the more quantitative methods of tracer study. In spite of many reports which have untilized newer concepts of photography and biology[4, 5, 7, 8] radioautographs have remained a somewhat imperfect tool. On the other hand, the modern day emphasis on histochemistry has caused many to turn hopefully again to the possibility of visualizing the location of radioactive elements in tissue. It is generally felt that the problems inherent in radioautography can best be solved by fitting them to the requirements of each experiment.

An analysis of the problems of obtaining satisfactory radioautographs with Ca^{45}, P^{32}, Sr^{89}, and other shorter lived isotopes such as F^{18} presented three main difficulties. The first and most formidable was the short half life of one of the isotopes to be used (112 min. F^{18}). This necessitated developing a technique which would reduce the time consumed between obtaining the sample and placing the section against the film to one hour or less. The procedures employed by Axelrod[2, 3] and Arnold[1] are too time consuming to be of any use in this problem. The same also applies to the use of methacrylates as described by Puckett[11], Sognnaes, Shaw, Solomon and Harvold[13] and Hall and Irving[9]. Furthermore, partial polymerization of methyl methacrylate is too exacting, time consuming, and expensive to give it widespread use. Smooth, flat sections are a requisite of good radioautography and the usual tearing and distorting of the soft tissues in a ground section might prove burdensome if not eliminated. Finally, it is difficult to determine which points on the original ground section correspond to the blackened areas on the radioautographs. One possibility which presents itself is the concurrent use of a roentgenogram and radioautograph of the undecalcified section. With these considerations in mind, the following objectives were established:

The development of a rapid method of fixing, dehydrating, and embedding of specimens consisting of soft and hard tissues to enable radioautographs to be made with short-lived isotopes. No reagents could be employed capable of causing a marked shifting of the metabolites to be studied. Furthermore, the technique generally used (that of obtaining undecalcified sections which were smooth and flat enough to be placed directly against a photographic emulsion for the purpose of obtaining radioautographs) had to be improved. Also a better means of orienting the betagraph and the section was highly desirable.

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The attainment of these technical objectives can best be divided into three steps:

1. Fixing and dehydrating.
2. Embedding and polymerization.
3. Sectioning and radioautographs.

In order to provide time for the initial polymerization 0.4 mm. sheet lead is molded into an open box which is one and a half times larger than the specimen. The floor of the box is coated with a thin layer of resin which is polymerized. This allows the specimen to be covered by resin on all sides.

Fixing and Dehydrating.—Fixing and dehydrating are obtained with dioxan.[10, 13] This introduces an unavoidable amount of leeching and transpositioning of the lipoid soluble fraction of phosphorus. A total of 10 times the volume of the specimens is used. The length of time for fixing and dehydrating varies with the size and nature of the material. Periods of 12 hours to 1 week have given satisfactory results. When smaller volumes of dioxan are used, 5 or 6 changes become necessary. Prior to embedding, the tissues are dried in an incubator at 50° C. to remove the dioxan.*

Embedding and Polymerization.—In the search for an adequate embedding material we were directed to R-250 Astrolite.† This is a polyester type resin which is available as the partially polymerized syrup with a shelf life of 1 year when kept in a cool dark place. Final polymerization is attained by using the proper number of drops of the accompanying peroxide catalyst. The number of drops added depends on the thickness and total volume of the material to be used. For molds 2 inches thick, 2 drops per 5 c.c. may be used, while for 1 inch molds 4 drops per 50 c.c. of plastic are indicated. The difference here is due to the poor heat conduction of the material. Thicker samples receive less accelerator in order to reduce the detrimental effects of the exothermic reaction which occurs. The peroxide catalyst and resin must be thoroughly mixed by stirring following which air bubbles are permitted to rise for a few minutes. The lead box containing the specimen is filled with plastic and placed in a heavy glass desiccator which is connected with a Duo Seal vacuum pump. A vacuum of 27 to 28 inches of Hg is produced and maintained for 5 minutes, then broken. This process is repeated until no air bubbles are seen to rise from the specimen as the vacuum is increased.

For isotopes with half lives greater than 1 day the polymerization process is facilitated by placing the mold in an oven at 80 to 90° C. for 8 to 10 minutes. Allowing the mold to stand at room temperature overnight completes the solidification. At this point the lead sheeting may be stripped away and set aside for reuse. The block now has a leathery consistency and a sticky surface. This is eliminated by a case-hardening process which consists of placing the block in an oven at 110° C. for 10 to 30 minutes. Since this procedure takes approximately 24 hours, which is too long for work with short-lived radioisotopes, a more rapid method of polymerization had to be developed. For this purpose one drop

*Since the work with F^{18} has been limited to intact teeth, fixing, and dehydrating were not necessary.

†From the Industrial Plastics Service, Oakland, Calif.

of one more catalyst, a metallic accelerator,* was added to the previously mixed resin and peroxide catalyst. This is then placed in an oven of 60° C. and polymerization occurs in about 15 to 20 minutes. The specimen is ready for sectioning approximately 30 minutes after the start of operation. The metallic accelerator and peroxide catalyst should not be mixed directly with each other as a danger of spontaneous combustion exists.

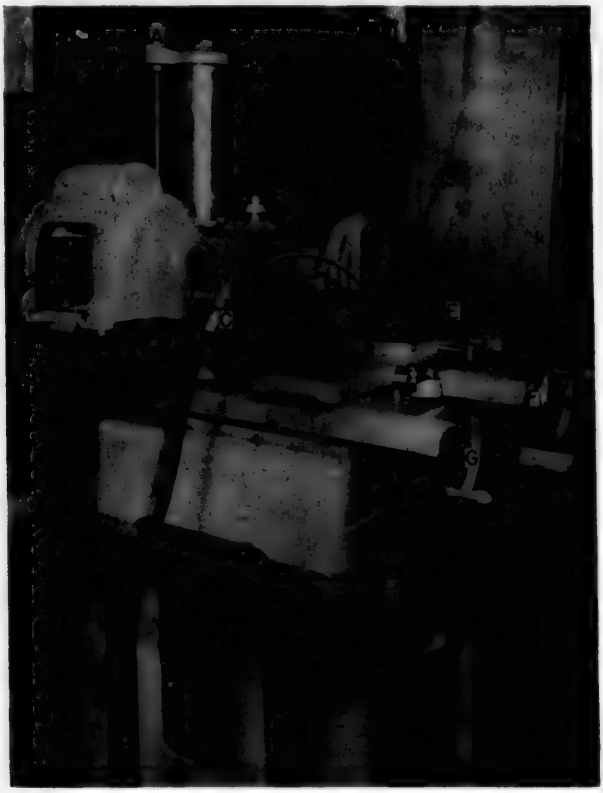

Fig. 1.—Modified Delta Surface Grinder used to obtain smooth flat ground sections of uniform thickness. The specimen in block of plastic is clamped in vise (E). Vertical depth of cut is controlled by vertical feed mechanism at (A) and micrometer tilting device at (B). Thickness of section is governed by traverse feed control (G) which moves specimen in forward and back direction. Longitudinal feed control (F) carries block in right and left direction from cutting wheel (D). Throughout operation coolant flows through tubing (C) to be sprayed against wheel. Reflected spray is collected in splash tray (H) from which it is returned to pump near floor to continue cycle. Not shown is transparent oiled silk curtain used to eliminate upward and lateral spray from wheel.

Sectioning and Radioautographs.—The sectioning of the block is done with a Delta Surface Grinder (Fig. 1) modified to receive a 7 inch diameter crystolon wheel of 0.525 mm. thickness. A two step pully permits speeds of 3200 or 4200 r.p.m. A pumping and collecting device is attached to the machine to provide a steady stream of soluble oil and water while cutting. Contamination from the spray is kept down with a piece of oiled silk arranged in the manner of a shower

*Available from Industrial Plastics Service, Oakland, California.

curtain. Sections are obtained from this machine with a smooth, flat surface, the soft tissue showing no tearing or distortion. Section thickness depends on the length and depth of the cut. Sagittal sections of a skinned rat head, for instance, can be consistently cut at a thickness of 700 microns. Thinner sections can be cut of specimens of smaller size. Uniformity of thickness and smoothness of the cut surface are of considerable value in the combined radioautographic and roentgenographic techniques.

Fig. 2.—A combined radioautograph and roentgenogram of a sagittal section of a rat head made with Ca^{45}. The dark black areas represent the site of the radioactive element while the radiopaque portion represents the roentgenographic shadow of those portions of the specimen which have taken up no Ca^{45}.

The sections are then glued on a piece of paper and placed against the film in a film box, the corners of which are reinforced with photographic tape. Close adaptation of the section to the film is secured by filling the space of the box with sponge rubber of sufficient thickness to give resistance to closing. Flatness and uniform thickness of the sections are important for good adaptation. No pressure or chemical effects on the emulsion have been observed. The closed box is kept together by heavy rubber bands and any potential light leaks are covered with photographic tape. Exposure time is calculated from the chart of Wainwright, Anderson, Hammor and Lehman[15] or by the method of Hamilton and Axelrod.[2] Activity estimates are made on a Tracerlab autoscaler with a

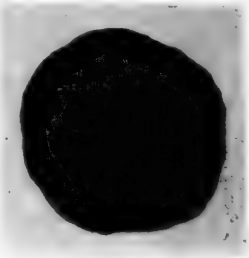

Fig. 3.—F^{18} radioautograph and roentgenogram of a cross section through the crown of a human premolar.

one inch diameter bell-shaped Geiger tube. Window thickness is 2 mg. per square centimeter. For long exposure times the box is refrigerated to reduce the fading of the latent image.[6, 12] Industrial Type A x-ray film is used which has optimal sensitivity to both the beta rays from the section and the x-rays which orient the effect of the beta rays.

Immediately prior to developing, the entire unit is given an exposure of 2 Ma of x-rays for 1 and one quarter seconds.[14] The radioautograph can then be studied in its relation to an outline roentgenogram (Fig 2). This is of considerable aid in evaluating the betagrams as it avoids having to use separate films for comparison. Buck's X-ray Developer is employed for 5 minutes and the film is fixed with Eastman Fixer and Replenisher for a minimum of twice the developing time.

A roentgenogram and F^{18} radioautograph of a cross section through the crown of a human premolar is shown in Fig. 3. The F^{18} can be identified by the blackened areas at the periphery of the enamel. A portion of the occlusal enamel is present in this section and may be seen through the dentin. The black dots represent the F^{18} accumulated in the pits of the occlusal surface.

SUMMARY

The description of a rapid fixation-dehydration and embedding method has been given which can be employed to obtain very satisfactory combined radioautographs and roentgenograms from sections of soft and hard tissues.

Some modifications of previous techniques have been developed which require minimal amounts of time with the view of applying them to studies with short-lived radioisotopes.

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THE COMPARATIVE EFFECTS OF PENICILLIN, BACITRACIN, CHLOROMYCETIN, AUREOMYCIN, AND STREPTOMYCIN ON EXPERIMENTAL DENTAL CARIES AND ON CERTAIN ORAL BACTERIA IN THE RAT

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PREVIOUS studies have indicated that the development of dental caries in white rats[1-4] and in hamsters[5] is significantly inhibited by penicillin. Other experiments have shown an inhibitory effect of aureomycin but no inhibitory effect of tyrothricin.[5] The clinical studies of Hill and Kniesner[6] failed to show any caries reduction in children from the unsurpervised use of a penicillin dentifrice whereas the clinical study of Zander[7] indicated that a significant reduction in caries activity occurred in children using a penicillin dentifrice under supervision at school.

In white rats McClure and Hewitt[1] found the oral lactobacillus counts to be markedly reduced by penicillin added to the drinking water and diet. On the other hand, Fitzgerald, Zander, and Jordan[8] found no significant reduction of salivary lactobacillus counts in children using a penicillin dentifrice for 18 or 24 months.

While the effectiveness of the antibiotics in reducing the development of caries is probably related to their antibacterial action, there is as yet no clear-cut evidence relating changes in the oral microflora to caries inhibition by these agents. It seemed desirable, therefore, to study several antibiotics which have different antibacterial spectra for their ability to prevent experimental caries in rats, and at the same time to follow the changes in selected groups of the oral bacteria of the animals. The antibiotics selected included penicillin and bacitracin which chiefly inhibit gram-positive bacteria, and streptomycin, aureomycin, and chloromycetin which inhibit in varying degrees both gram positive and gram negative microorganisms.

MATERIALS AND METHODS

The plan of the experiments was as follows. Littermate rats were evenly distributed into control and test groups. Twenty-four rats representing 24 different litters were placed in each group. Because of limitations in the size of litters, the experiments were divided into two parts; the first part tested the effects of aureomycin and penicillin, while the second part tested the effects of chloromycetin, streptomycin, and bacitracin. The rats, all females, were of the Sprague-Dawley strain, 20 to 22 days old, taken at weaning from the NIH stock

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colony. This stock colony is fed Purina Laboratory Chow, kale leaves, and water ad libitum. Each animal was kept on the experiment in individual screened bottom cages. Food and water consumption were ad libitum.

The basal diet to which the antibiotics were added was as follows:

(DIET 585)

Dried whole milk powder	30%
Yellow hominy corn grits (Quaker)	42%
Cane sugar—granulated	25%
Whole dried liver substance (Wilson)	2%
Salt Mixture*	1%

*Salt Mixture:

Nacl	400 parts
KCl	400 parts
$MgCO_3$	100 parts
Ferric Ammonium Citrate, Brown, USP	20 parts
Manganese Sulfate · $4H_2O$	28 parts
$CaHPO_4$	50 parts
Cupric Acetate · H_2O	2 parts
Total	1000 parts

The salts were mixed and ground to fine powder (with mortar and pestle). In addition, raw carrots were given one day a week.

This diet was initially selected from several diets which were tested in auxanographic culture plate experiments because it best supported the growth of acidogenic oral bacteria. Also, in previous experiments with rats it had proved highly cariogenic, i.e., out of 150 rats fed this diet 149 developed severe occlusal fissure caries. In the present experiments this diet produced caries in 100 per cent of the control animals.

The experimental diets containing the required amounts of the different antibiotics were prepared semi-weekly. The quantities of antibiotics per 100 grams of food were as follows:

Na Penicillin-G	50,000 units per 100 grams
Bacitracin	5,000 units per 100 grams
Aureomycin·HCl	50,000 micrograms per 100 grams
Chloromycetin (chloramphenicol)	50,000 micrograms per 100 grams
Streptomycin-$CaCl_2$	50,000 micrograms per 100 grams

All diets were kept in cold storage.

Prior to starting the experiment, microbiologic assays showed that excepting the penicillin diet, all the antibiotic diets remained stable after standing in the food cups for at least one week. The penicillin diet gradually deteriorated after standing 72 hours. This latter diet was renewed, therefore, every 48 hours whereas all the other diets were renewed every 2-3 days as required.

Bacteriologic studies of the oral flora were made before and during the course of the experiment. The rats were fasted overnight prior to these studies.

Accessible molar tooth surfaces were swabbed with a sterile cotton-tipped stick which was cut off into a sputum bottle containing 10 ml. of a 0.05 per cent aqueous yeast extract. The bottles were then allowed to stand in a cold room for two hours. Dilutions for plate counts were made after the bottles had been shaken for two minutes on a shaking machine.

Lactobacillus plate counts were made on the specific lactobacillus medium of Rogosa[9] and on pH 5.0 tomato juice agar to which had been added 20 μg. polymyxin sulfate per milliliter to inhibit gram-negative bacteria. Streptococcal

counts were made in crystal violet azide agar containing 5 per cent defibrinated sheep blood. Coliforms were counted on eosin-methylene blue agar. All plates were incubated aerobically at 37° C.

At the end of approximately 122 days the animals were sacrificed, the heads autoclaved and cleaned of soft tissues. The molar teeth were examined microscopically at magnifications of 10× and 30× using a sharp dental explorer and bright illumination. Independent examinations were made by two of the authors after which agreement was reached on a common diagnosis. The teeth were also scored according to the systems of Cox, Dodds, Dixon, and Matuschak.[10]

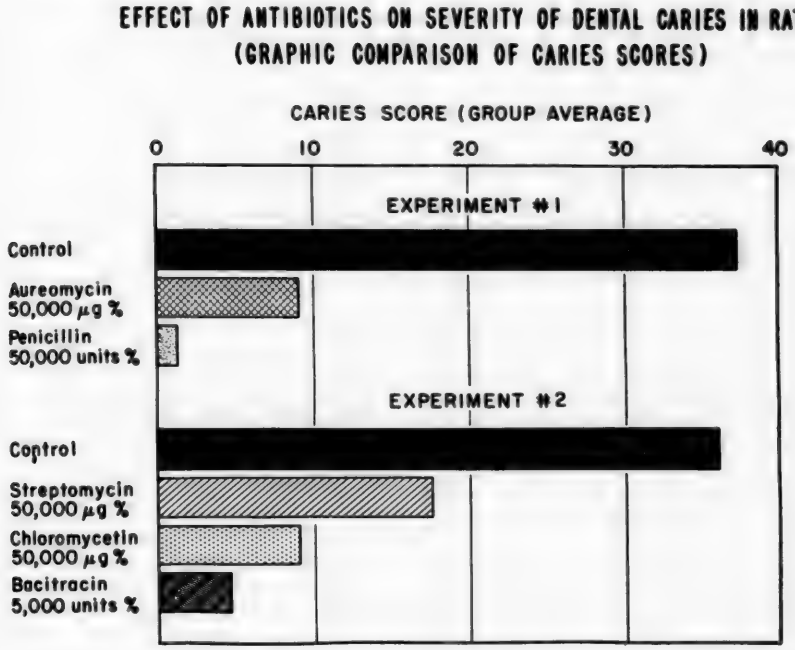

Fig. 1.

RESULTS

Effect of Antibiotics on Dental Caries.—The essential data relative to the effect of the antibiotics on caries in these rats are given in Table I. The relative effects of the different antibiotics upon the extent of the caries (caries score) is also shown graphically in Fig. 1.

The reduction in caries brought about by the various antibiotics is evident in (1) the per cent of rats which developed caries (2) the number of teeth which became carious and (3) the caries score (which attempts to evaluate the extent of each carious lesion). By all of these criteria, penicillin showed the greatest inhibitory effect. Bacitracin was next in order of effectiveness, aureomycin and chloromycetin produced about equal inhibitory effects, while streptomycin pro-

duced the smallest reduction in caries. While all of the rats given streptomycin developed caries, the severity of the caries (caries score) was only about half as great as the caries which developed in the control animals.

During the course of scoring the teeth for caries, it was observed that many animals in the antibiotic groups showed some fracturing of the enamel on the occlusal cusps without visible evidence of caries in the occlusal fissures. On the other hand, in the control groups similar fractures were generally associated with definite evidence of caries deeper in the occlusal fissures.

TABLE I

THE EFFECT OF ANTIBIOTICS IN A CARIOGENIC DIET

GROUP	A	B	C	AA	D	E	F
Antibiotics in diet	None	aureomycin	Penicillin	None	Bacitracin	Chloromycetin	Streptomycin
No. animals surviving experiment	23	23	24	24	23	23	22
Initial weight of animals —average (grams)	31	31	32	32	30	32	31
Final weight of animals —average (grams)	203	220	214	220	213	208	202
Estimated average daily food consumption per rat (grams)	10.1	11.1	10.1	10.9	9.1	9.7	9.9
Estimated average daily antibiotic consumption per rat	--	5550 μg.	5060 units	--	456 units	4830 μg.	4970 μg.
No. of rats with caries	23	21	4	24	14	22	22
Per cent of rats with caries	100	91	17	100	61	96	100
Total No. carious teeth per group	125	65	14	118	41	67	76
No. carious teeth per rat (average)	5.4	2.8	0.6	4.9	1.8	2.9	3.5
Per cent reduction in No. carious teeth (compared with respective control)	--	48	89	--	64	41	30
Average carious score per carious rat	37.4	9.8	7.6	36.0	7.8	9.6	17.6
Average carious score per rat per group	37.4	9.0	1.3	36.0	4.7	9.2	17.6
Per cent reduction in carious score compared to control	--	76%	96.5%	--	87%	74%	51%

A summary of the bacteriologic data is presented in Table II. In this table the counts done on individual rats are expressed in group averages. Lactobacillus and coliform counts were made on all the animals at the start, at about the middle (50 days), and just before the end of the experiment (110 days). Streptococcus counts were made at the start and after 72 days. When the data were considered in terms of the relationship of individual bacteriologic findings to individual caries scores it was not possible to demonstrate any definite pattern relating the two. Even when group averages were used as in Table II the same lack of correlation was evident. For example, Chloromycetin which gave a general reduction of lactobacillus counts was equally effective in controlling caries as aureomycin which had little effect on the lactobacillus counts. On the other hand the

effect of the two drugs on the oral streptococcal counts was exactly the reverse. With penicillin and bacitracin both the lactobacillus counts and streptococcus counts decreased along with the caries score.

TABLE II
A SUMMARY OF BACTERIOLOGICAL FINDINGS*

	LACTOBACILLI			STREPTOCOCCI		COLIFORMS		
	DAYS ON EXPERIMENT			DAYS ON EXPERIMENT		DAYS ON EXPERIMENT		
	0	50	110	0	72	0	50	110
A Controls	3,300	1,100	9,800	452,000	290,000	1,600	2,280	2,340
B Aureomycin	2,200	1,800	14,000	499,000	5,950	2,050	69,340	16,020
C Penicillin	1,800	260	1,780	536,000	19,000	1,825	18,950	5,110
AA Controls	5,300	1,500	21,960	391,000	207,000	1,200	9,400	2,270
D Bacitracin	5,300	470	180	412,000	14,500	1,850	10,870	1,770
E Chloromycetin	6,500	120	845	330,000	485,000	1,250	14,420	2,050
F Streptomycin	6,900	1,475	13,800	428,000	37,000	1,130	17,320	18,380

*All counts are averages of the individual counts for each animal in the group and represent the average number of organisms per swab.

Usually no attempt was made to identify the various organisms further than the general groupings on the basis of colony characteristics on the special media employed. At various times during the course of the experiment over one hundred separate colonies of lactobacilli were isolated, cultured in vaspar-sealed stabs of Rogosa's medium and observed for gas production. In this way it was determined that about 80 per cent of the oral lactobacillus population of the rats on this diet were of the heterofermentative type. The vast majority of the streptococci were of the alpha or gamma type on blood agar. About 50 such strains were tested in glucose broth and all gave positive methyl red tests indicating the ability to produce a pH of below 5.0. On the E.M.B. plates colonies presumptive of *E. coli* made up only one tenth or less of the total in the control diets, most of the colonies presenting the appearance characteristic of *A. aerogenes* on this medium. On the penicillin and bacitracin diets the proportion of *E. coli* to total coliform types increased, while with the streptomycin, aureomycin, and Chloromycetin diets the proportion of *E. coli* types decreased.

DISCUSSION

The results of these experiments are in general agreement with and extend the scope of previous findings in indicating that the development of occlusal

fissure caries in experimental animals on coarse particle diets may be inhibited by the use of antibiotic agents. The experiments reported here furnish a relatively rigorous test of the effectiveness of the antibiotics because the diet employed induced severe caries in all of the control animals. The fact that none of the antibiotic groups was completely free of caries at the comparatively high levels of antibiotics which were used, suggests that the effectiveness of the antibiotics in inhibiting experimental caries in rats depends somewhat on the caries producing qualities of the diet and on other factors as yet undetermined.

It may be of some significance that the antibiotics which specifically inhibit the gram positive flora (namely, penicillin and bacitracin) proved most effective in preventing caries. Aureomycin, Chloromycetin, and streptomycin which possess a wider spectrum but are less specific for oral streptococci or lactobacilli in vitro were less effective in reducing caries in vivo. However, from the bacteriological findings obtained in the current study it is difficult to assess this point with any degree of certainty. Moreover, it should also be pointed out that the present study considered only a small part of the oral flora cultivable on aerobic plates, leaving the significance of changes in the remainder of the aerobic micro flora and the equally numerous anaerobic microflora still to be ascertained.

The demonstrated ability of a number of antibiotics with different antibacterial spectra to inhibit the development of experimental caries to different degrees is of further interest. Extension of studies of this type may furnish a means of defining more precisely the bacterial etiology of dental caries. It may also be possible to reduce the numbers of unassociated microbial variables by the use of suitable antibiotics, and thus aid in clarifying the caries picture.

SUMMARY

The antibiotics penicillin, bacitracin, aureomycin, Chloromycetin, and streptomycin were studied for their effectiveness in reducing experimental dental caries in the albino rat by adding them to a coarse particle diet. Penicillin at a concentration of 50,000 units per cent was most effective followed by bacitracin (5,000 units per cent), aureomycin, Chloromycetin, and streptomycin all at 50,000 micrograms per cent.

Studies of oral lactobacilli, streptococci, and coliforms revealed no consistent changes relatable to the caries picture.

We gratefully acknowledge the technical assistance of Mr. William L. Poole and Mr. Frank R. Shaw.

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EFFECTS OF SONIC VIBRATION ON THE NUMBERS OF MICRO-ORGANISMS CULTIVABLE FROM HUMAN SALIVA

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QUANTITATIVE estimates of the cultivable micro-organisms in pure cultures, water, milk, foodstuffs and the secretions and excretions of human beings are influenced by the physical arrangement and dispersion of the organisms in the samples. It has become common practice, therefore, to shake the sample, with or without glass beads, by hand or mechanically for varying periods of time prior to culturing, in order to obtain a more homogeneous dispersion, so that counts of colonies will closely indicate the total cultivable content. The application of shaking procedures to saliva, or washings from the oral cavities of human beings, has encouraged many workers to carry out a variety of studies on the oral microbial flora. Most workers have not been satisfied, however, that the counts obtained were representative of the cultivable micro-organisms in the samples, even though the counts were suitable for the purposes of the particular study. Several investigators have described methods, including, or apart from shaking, for obtaining more homogeneous suspensions. Crowley and Rickert[1] passed saliva through an atomizer, Bibby and Maurer[2] treated saliva with horse serum to prevent clumping, while Permar, Kitchin, and Robinson[3] studied a variety of methods.

The purpose of this report is to describe our findings after exposing saliva to sonic vibration (9,000 cycles per second) prior to dilution and distribution on medium in plates. The use of sonic vibration was suggested by the results of turbidity measurements with a Klett-Summerson photelometer, before and during sonic vibration of a suspension of lactobacilli in an effort to disintegrate the cells. During the first 15 minutes of exposure there was an increase in turbidity (an increase in the number of light-absorbing particles) which, during the next 15 minutes, began to decrease as the cells disintegrated. Since smears of this suspension prior to sonic vibration showed "palisade" arrangement and clumping, it was presumed that the increase in turbidity was due to a dispersion of cell aggregates, similar to the observation reported by Shropshire.[4] No smears were made during sonic vibration but smears at the end of treatment indicated a homogeneous suspension of disintegrated, partially disintegrated and whole cells. If the increase in turbidity was due to a separation of the cell aggregates into smaller units, one might expect a higher colony count on culture medium from a suspension treated for a particular time interval than from an untreated one.

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MATERIALS AND METHODS

Collection of Saliva.—A piece of sterile paraffin (M.P. 50 to 52° C.) was allowed to soften in the mouth and then chewed for 3 to 5 minutes. As saliva collected it was transferred to a sterile bottle. Saliva samples were collected from 4 persons and pooled for all work involving the use of more than 10 ml. of saliva. The various samples of a pooled saliva employed in these studies were not made up of saliva collected from the same persons.

Treatment of Saliva.—The methods for treating saliva are all mechanical and differ only in the amount of energy applied and the frequency of application. For the sake of convenience, however, the methods are designated as follows.

a. Sonic vibration: The apparatus employed was a Raytheon magnetostrictor (Model #R-22-1) with a frequency of 9×10^3 cycles per second and a power output of about 50 watts. The transducer was removed prior to each run and autoclaved for 15 minutes at 120° C. A control on the sterility of the transducer cup was made prior to each run by adding sterile saline (0.85 per cent NaCl) to the cup after autoclaving, then exposing to sonic frequency for 2 minutes and culturing. Results were uniformly negative.

Saliva was transferred to the sterile cup, observing aseptic precautions, and exposed for varying time intervals. Aliquots for culture were removed periodically by opening the cup and removing 1.0 ml. with a steril 1.0 ml. pipette.

b. Mechanical shaking: A special shaking machine designed to deliver 85 cycles per second was employed (the platform of the machine traversed a distance of $\frac{3}{16}$ of an inch in each $\frac{1}{2}$ cycle). Saliva, usually 15 ml. was transferred to a sterile vial filled with glass beads (3 to 4 mm. in diameter) and closed with a sterile screw cap. The vial was fixed on a platform attached to the machine and the machine was run for varying time intervals. Aliquots were removed in the same fashion as for sonic vibration.

c. Mixing with pipette: A 5 ml. pipette (delivery aperture about 1 mm. in diameter) was employed to mix the saliva by withdrawing and expelling it forcefully 20 times within the confines of the container before aliquots were removed for culture.

Culture Methods.—The standard aliquot of 1.0 ml. was diluted serially in nutrient broth (Difco) so that dilutions of 1×10^{-1} through 1×10^{-6} were obtained. All transfers between dilutions were made with 1.0 ml. of the saliva broth mixture using a 1.0 ml. pipette. A 0.1 ml. pipette was used to withdraw 0.1 ml. from the appropriate dilutions for transfer to culture medium in a Petri plate. The aliquot was expelled onto the surface of the medium then spread over the surface by means of a sterile "L" shaped glass spreader. Plates were incubated aerobically at 37° C. for 72 hours. The media employed in the various phases of the study were:

1. Difco blood agar base plus sterile horse blood to a concentration of 5 per cent.
2. Blood agar (as in 1.) plus 0.01 per cent sodium azide and crystal violet 0.0002 per cent.[5]
3. Tomato juice agar pH 5.[6]

Counts.—All plates, with cover removed, were placed on a ruled card (Frost) on the stage of a binocular dissecting microscope. The 0.7 × objective was routinely employed for counting colonies on the surface of the medium, while

the beam of a microscope lamp was directed at the surface of the medium. Indirect light was used if necessary. Effort was made to count only those plates which presented 100 to 300 colonies on the surface of the medium.

RESULTS

A study to compare the effects of mechanical shaking and sonic vibration on the numbers of bacteria cultivable from pooled saliva was undertaken prior to making observations on the effects of sonic vibration alone. A 50 ml. amount of pooled saliva was mixed thoroughly, using a pipette, then 1.0 ml. was removed for dilution and culture. The counts made from the appropriate dilution after pipette-mixing were referred to as counts at zero time. A 15 ml. portion was treated by mechanical shaking while the remainder was exposed to sonic vibration. Aliquots of 1.0 ml. were removed from the portions treated by these methods at intervals of 2, 6, 10, 15 and 30 minutes. Plates were made from several dilutions but the 10^{-4} dilution usually yielded the best distribution of colonies for "zero time" counts as well as those after mechanical shaking, while the 10^{-5} dilution was best after sonic vibration. A total of 5 Petri plates, containing blood agar, were inoculated from the appropriate dilutions from each aliquot.

The average number of organisms from each series of plates are recorded in Table I, while Fig. 1 is a graphic presentation of these data. The plates

TABLE I
COMPARISON OF THE EFFECTS OF MECHANICAL SHAKING AND SONIC VIBRATION ON COUNTS OF BACTERIA IN SALIVA

TIME IN MINUTES	NUMBER OF BACTERIA × 10^6/ML. POOLED SALIVA		
	Pipette	MECHANICAL SHAKING	SONIC VIBRATION
0	23*	—	—
2	—	21	114
6	—	24	105
10	—	†	196
15	—	23	117
30	—	26	151

*Counts represent average number of colonies appearing on 5 blood agar plates made at each time interval.
†Plates contaminated.

inoculated from the dilution of the 10 minute aliquot from the sample treated by mechanical shaking were contaminated. Comparison of the average counts after mechanical shaking with the average count following pipette-mixing indicates that the former treatment did not lead to appreciably higher counts. On the other hand, sonic vibration led to markedly higher numbers of colonies. Exposure of the sample for only 2 minutes led to a count that is 5 times higher than the average count after mechanical shaking of the same sample. The count was not changed appreciably after exposing for 6 minutes, but the maximum count for this sample was observed after vibrating the sample for 10 minutes. The count after 15 minutes of exposure was lower than after 10 minutes and is noted to be at the level of the 2 and 6 minute counts. A relatively higher count was found after 30 minutes than after 15 minutes. Statistical analysis is limited to

a comparison of the means of counts resulting from the various methods of treatment and is based on chi square tests which show that the colonies on the various plates occurred in a Poisson distribution. Comparison of the mean count after pipette-mixing with the various mean counts after mechanical shaking indicated that there was no particular difference in the populations of samples removed. Similar results were found upon comparison of the various mean counts after the different intervals of mechanical shaking. However, the differences between the mean count after pipette-mixing and the mean counts after each interval of sonic vibration, as well as the differences between mean counts after mechanical shaking, and those after sonic vibration, are significant at the 1 per cent level (all P values are less than 0.01). The analyses indicate a definite difference in the dispersion of cultivable bacteria after sonic vibration in contrast to mechanical shaking or pipette-mixing.

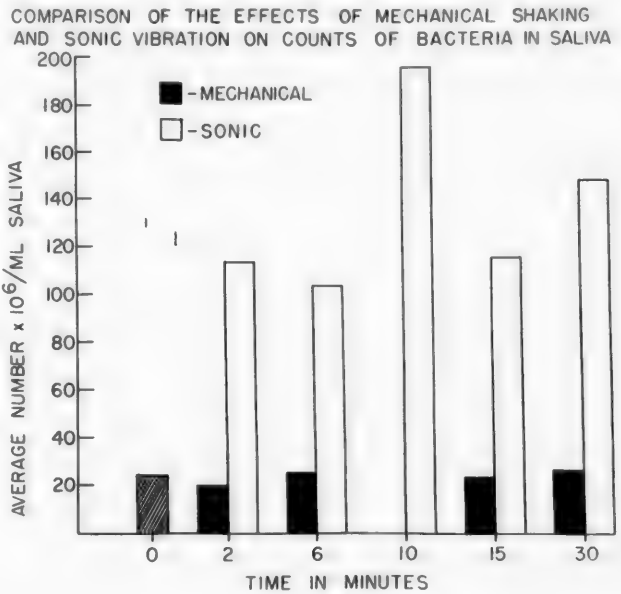

Fig. 1.

Comparison of the mean counts after different time intervals of sonic vibration was made to determine whether the observed differences were significant. The difference in the mean counts after 2 and 6 minutes does not reflect a significant change in the population of the samples removed. The differences between these mean counts and that after 10 minutes indicate a marked change in the samples since both P values are less than 0.01. The mean count after 15 minutes differs significantly from that after 10 minutes indicating a reduction in the population of the samples. The higher mean count after 30 minutes was not expected. A comparison of these mean counts, however, indicates that the difference is significant at the 1 per cent level (P value is less than 0.01). These

analyses indicate there is little change in the population of the samples during the first 6 minutes of sonic vibration but significant changes occurred, during the run, after 10, 15, and 30 minutes.

A sample of saliva obtained from 1 person was employed to compare the effects of sonic vibration and mechanical shaking on the numbers of colonies when the sample was *diluted* before exposure to shaking methods. The sample was diluted 1:5, then 1 portion was exposed to mechanical shaking for 10 minutes and the other to sonic vibration for 5 minutes. Five blood agar plates were inoculated from the 10^{-4} dilution of the aliquot from the sample treated by mechanical shaking, a similar number were prepared from the 10^{-5} dilution of the sample exposed to sonic vibration. The time intervals employed in the treatment of this sample were arbitrarily set at 10 minutes for mechanical shaking and 5 minutes for sonic vibration without benefit of the analysis of the previous work with the pooled samples. The numbers of colonies on each plate, the arithmetic means of each group of plates, and the standard deviations of the counts are recorded in Table II and presented graphically in Fig. 2. It will be noted that the means and standard deviations are on the right hand side of Fig. 2. The range of the counts after mechanical shaking is slightly lower than the range of the counts after sonic vibration, but sonic vibration led to an average count that is almost 3 times higher than the count after mechanical shaking.

TABLE II
COMPARISON OF COUNTS FROM A SINGLE DILUTION FROM SALIVA SAMPLE DILUTED BEFORE MECHANICAL SHAKING AND SONIC VIBRATION

	NUMBER OF BACTERIA × 10^6/ML. SALIVA	
ALIQUOTS*	MECHNICAL SHAKING 10 MINUTES	SONIC VIBRATION 5 MINUTES
1	68	235
2	85	225
3	80	275
4	99	250
5	77	245
Mean	82	245
Standard deviation	10	17

*One plate per aliquot.

The variability in the counts is about as may be expected in random sampling since the values obtained from application of the chi square test are not unusual and the standard deviations squared (variance) are roughly equal to, although slightly higher than, the respective means. It is concluded that the colonies on the plates were in a Poisson distribution. A comparison of the means indicates that the difference is significant at the 1 per cent level (P value is less than 0.01). The data indicate that sonic vibration yields higher numbers of colonies from *diluted* saliva samples than mechanical shaking, thus these results are similar to those found after sonic vibration of undiluted saliva. A larger number of replicate plates would be needed in order to determine whether there is less variance in the counts after sonic vibration in contrast to mechanical shaking.

Exposure of saliva to sonic vibration at varying time intervals was repeated using 2 different samples of pooled saliva. The periods of exposure were the same as in the work described above except that observations were also made after 4, 45, and 60 minutes. Aliquots removed from the samples after each period of exposure, were diluted and cultured so that counts of colonies of streptococci and lactobacilli could be made in addition to the colony counts on blood agar plates. An inoculum of 0.1 ml. of appropriate dilution of each aliquot was placed on each of 2 plates of an agar medium described by Stiles and Chapman,[5] tomato juice agar,[6] and 5 per cent blood agar. The average of the counts from each pair of plates for each medium and each pooled saliva are presented in Table III. It will be noted that in some cases only 1 plate of a pair could be

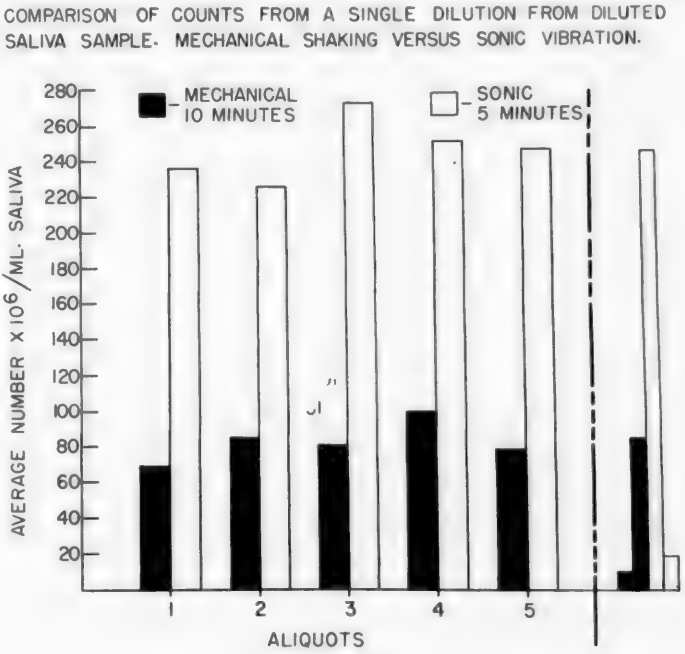

Fig. 2.

counted, whereas in others neither plate could be counted, because of contamination. If one plate was countable that count is given rather than an average. The data for the counts from blood agar plates, and the colony counts of streptococci and lactobacilli are presented graphically in Figs. 3, 4, and 5 respectively. No statistical analyses of these data were made because of insufficient information. Our main purpose was to study the trends in quantitative relationships between the various bacteria during exposure to sonic vibration.

The results from exposure of these 2 saliva samples to sonic vibration support the observations that this method of treatment increases the number of bacterial colonies appearing on culture media. The order of the increase is a

little lower than observed in the first study, for the counts at zero time in these samples are relatively higher. Fig. 3 shows that the average counts tend to increase to a maximum after 10 minutes, then decrease to counts after 60 minutes, that are lower than after 2 minutes. Saliva No. 1, yielded a higher average count after 4 minutes than after 10 minutes and Saliva No. 2 yielded a single count after 30 minutes that is higher than the average count after 10 minutes. These exceptions to the general trend cannot be evaluated because of the lack of sufficient replicate plates. The contamination of plates, inoculated from sample No. 2 after 15 minutes, prevents any comparison of the trend in the counts from this saliva sample after 15 and 30 minutes of sonic vibration with those shown in Fig. 1. However, it will be noted that the trend in the average counts in sample No. 1 does not indicate a lower count after 15 minutes than after 10 or a higher count after 30 than after 15 minutes.

TABLE III
EFFECT OF TIME OF EXPOSURE TO SONIC VIBRATION ON NUMBERS OF BACTERIA CULTIVABLE FROM SALIVA

	TIME IN MINUTES	NUMBER OF BACTERIA PER ML. SALIVA					
		SAMPLE NO. 1*			SAMPLE NO. 2		
		B	S	L	B	S	L
Pipette	0	81	34	138	87	41	12
Sonic vibration	2	213	82	180	251	133	21
	4	367	159	175	†	178	26
	6	305	147	156	247	171	31
	10	338	157	128	339	176	24
	15	292‡	193	199	†	210	29
	30	267	14	124	374‡	242	14
	45	198	9	73	240	99	8
	60	187	109	51	145‡	64	4

*Saliva pooled from 4 different persons. The second sample was from a different group.
†Plates contaminated.
‡Count from single plate.
B, Blood agar plate count—number × 10^6.
S, Streptococci—number × 10^6.
L, Lactobacilli—number × 10^3.

The results of the effects of sonic vibration on counts of streptococcus colonies (Fig. 4) in the 2 saliva samples indicate that the counts are consistently higher than those obtained after mixing with a pipette. The counts for the No. 1 sample are generally lower than for sample No. 2, but since the counts were made from pooled saliva it is not possible to attach any significance to the differences. The trend of the streptococcus counts over the entire observation period is different from that in Fig. 3. The counts after 4 minutes of exposure were not changed appreciably by exposure for 6 or 10 minutes. The streptococcus count for sample No. 1 is noted to be maximum after 15 minutes of exposure. Lower counts were observed for both samples after 45 minutes. Little change in the count for sample No. 1 was observed after 60 minutes, but the count from sample No. 2 is noted to have decreased compared to the counts at 45 minutes. The data indicate that the highest colony counts for these bacteria were not found until after 15 or 30 minutes of exposure to sonic vibration and in this

EFFECT OF TIME OF EXPOSURE TO SONIC VIBRATION ON NUMBER OF BACTERIA CULTIVABLE FROM SALIVA.

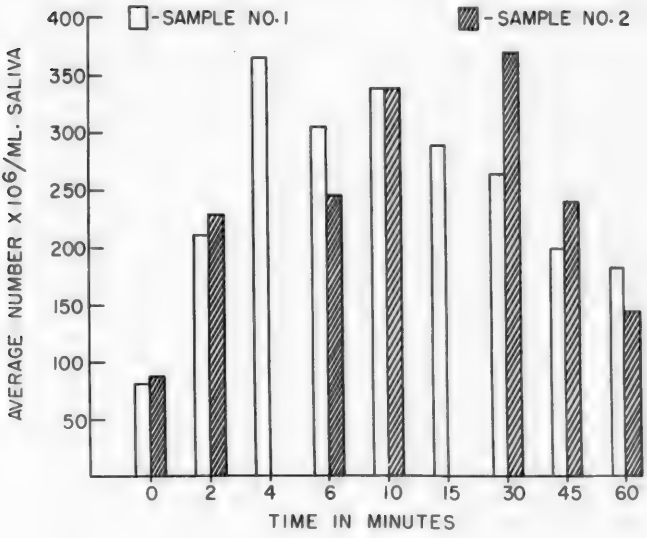

Fig. 3.

EFFECT OF TIME OF EXPOSURE TO SONIC VIBRATION ON NUMBERS OF STREPTOCOCCI CULTIVABLE FROM SALIVA.

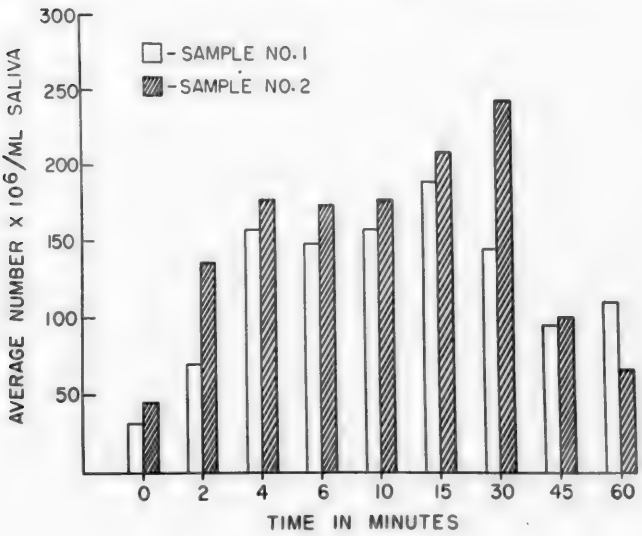

Fig. 4.

respect are different from the "total colony counts" for these saliva samples, but counts of streptococci as well as counts of all colonies on blood agar plates are noted to be lower after 45 and 60 minutes.

It is interesting that the counts of streptococcus colonies represent about half the numbers of colonies counted on blood agar plates ("total counts"). The proportion of colonies in sample No. 1 is over 50 per cent after 15, 30, and 60 minutes of sonic vibration, while over 50 per cent is noted at 2, 6, 10, and 30 minutes for sample No. 2. The data lend support to the statement that streptococci predominate in the cultivable oral flora.

The lactobacillus colony counts for samples Nos. 1 and 2 are presented in Table III and Fig. 5. There is a marked difference in the numbers of colonies from the 2 samples and the effects of sonic vibration on the counts also appear to differ. The average count for sample No. 1 is noted to be higher after 2

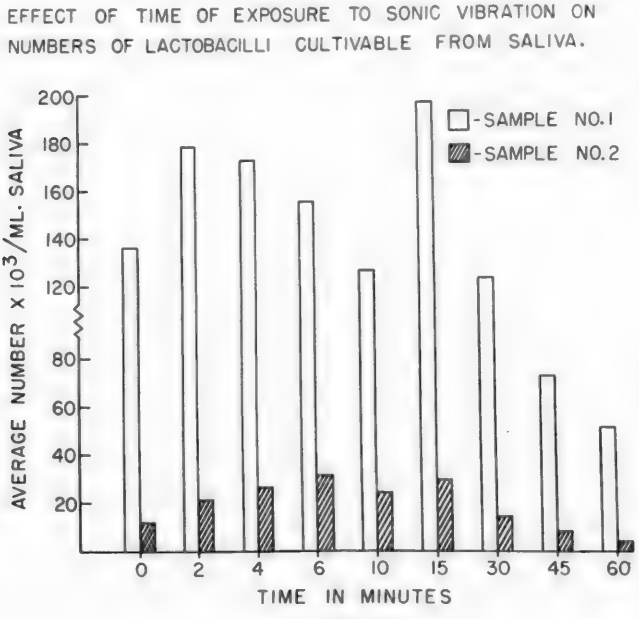

Fig. 5.

minutes of sonic vibration than the count at zero time. Each of the next 3 counts were progressively lower than the count after 2 minutes; then a maximum average count for the sample is noted after 15 minutes of exposure. The counts after 30, 45, and 60 minutes, respectively, were progressively lower than the maximum and lower than counts made at zero time or after 2 minutes of sonic vibration. Interpretation of these data is difficult because of the lack of sufficient replicate plates, but further work is indicated on salivas, preferably from one person, that contain relatively high numbers of cultivable lactobacilli.

The counts from sample No. 2 indicate relatively lower numbers of lactobacilli than in sample No. 1. It is noted in Fig. 5 that an increase in the average

count is apparent after 2 minutes of sonic vibration; the average counts at 4 and 6 minutes are higher, with the maximum number being found after 6 minutes of treatment. There is a lower average count after 10 minutes followed by a near maximum after 15 minutes. Progressively lower average counts were found after 30, 45, and 60 minutes respectively. The data indicate that the average counts in sample No. 2 were at least doubled after sonic vibration for between 4 and 15 minutes.

DISCUSSION

Early interest in the use of sonic or ultrasonic vibration seems to have been centered on the lethal effects on bacteria, therefore, attention was directed toward determination of the shortest exposure time which would kill the largest numbers of bacteria. As a result, little work has been done to determine what effects nonlethal exposure times might have on suspensions of bacteria. Shropshire[4] exposed suspensions of pure cultures of bacteria for short periods and reported that there was a breaking up of clumps and a relatively uniform dispersion of the forms, but as the time of exposure was extended there was a progressive disintegration of the cells. On the other hand, as far as we are aware, he did not report on cultural studies during these intervals. Our data indicate that exposure of saliva to sonic vibration altered the relative dispersion of the cultivable bacteria in the samples so that after short time periods consistently higher colony counts could be obtained than by a mechanical shaking method. Results somewhat similar to ours have been reported by Hopwood,[8] Hamre,[9] and Newton[10] after treatment of viruses. When certain viruses were exposed to frequencies of 5.5×10^5 cycles or higher the number of infective units could be increased. These workers attributed the increases to a dispersion of aggregates of the micro-organisms, but Newton[10] added that when tobacco mosaic virus was exposed to higher energy levels there was a fracturing of the basic infective unit.

The lethal effects of sonic or ultrasonic vibration on micro-organisms depend upon the kind of micro-organism,[11] the suspending fluid, the concentration of the organisms,[12] the time of exposure and the energy applied.[9] Beckwith and Weaver[11] found that the protein in milk was responsible for a diminished lethal effect of sonic energy on bacteria, while Rivers, Smadel, and Chambers[13] reported prevention of inactivation of elementary bodies of vaccinia when virus was suspended in serum. Our data indicate indirectly that saliva reduces the lethal effect of sonic vibration on bacteria, e.g., the numbers of colonies of streptococci cultivable from saliva (Fig. 4) were reduced by about two-thirds after one hour of exposure, whereas a much greater reduction might be expected if these organisms had been suspended in broth or physiologic saline.[4]

The highest numbers of colonies on the plates inoculated from the samples employed, within the limits of the media and incubation procedures used, were found after exposure to sonic vibration for about 10 or 15 minutes. The effects of exposure on counts made at time intervals between 6 and 10 minutes or 15 and 30 minutes have not been explored. The data do not offer any way of explaining the significantly higher average count after 30 minutes in comparison with the 15 minute count as shown in Fig. 1. A lethal effect might account for the lower

count after 15 minutes, but a postulation of a disaggregation of a different group of bacteria from those cultivated after the maximum count, or a fracturing of bacteria, seems necessary to account for the counts observed after 30 minutes. Simultaneous "differential" counts might have yielded some of this information.

Determination of the relative numbers of different bacteria in a given volume of saliva as determined by colony count may yield information as to the quantitative relationships between these microbial forms. Hemmens and associates[14] indicated the value of more than one type of medium, or cultural conditions, when a variety of bacteria are to be cultivated from a particular sample. Their study was concerned with the incidence of microbial forms in dental plaques rather than quantitative estimates. Different kinds of cultivable bacteria in saliva have often been estimated on a single medium, but crowding and suboptimal nutritive conditions for certain forms complicate the method. Studies on the numbers of bacterial colonies arising on separate media after simultaneous inoculation from saliva samples have been reported recently by Slanetz and Brown.[15] One medium was employed to obtain a "total" bacteria count and the numbers of colonies of streptococci belonging to the viridans group. A separate medium was used for enumeration of lactobacillus colonies. Their work was primarily concerned with the changes in relative numbers of these bacteria before and after use of antibacterial agents in the mouth, rather than consideration of quantitative interrelationships among the different bacteria. Information such as this cannot be obtained from the data presented here because of the use of pooled saliva. However, preliminary studies on the possibility of making "differential" counts on bacteria in saliva samples indicate that streptococci, lactobacilli, fusiform bacteria and an unidentified gram-negative diplococcus are cultivable in sufficient frequency to be enumerated on separate media. The use of sonic vibration for treatment of the sample prior to cultivation is a definite advantage in isolation except for fusiform bacteria. It was found, in the only test conducted thus far, that counts of fusobacteria were markedly reduced after an exposure of the saliva for 5 minutes, but mechanical shaking did not affect the count.

Bibby and Maurer[2] estimated they could culture only about one-sixth of the bacteria in the saliva samples studied, but also noted that they recovered in culture a higher proportion than had been previously reported. Although no direct counts and simultaneous colony counts have been made on a saliva sample exposed to sonic vibration, the consistently higher counts than the shaking method suggests that under certain conditions sonic vibration may yield counts that approximate the actual viable population size in any given sample. The studies reported here are preliminary in nature, therefore, several other aspects in addition to the above application are of interest, i.e., the effects of different intensities of energy and varying frequencies on colony counts of various cultivable bacteria.

SUMMARY

1. A comparison of the numbers of colonies appearing on a blood agar medium after exposing separate aliquots of pooled saliva to sonic vibration (9,000

cycles per second, power output about 50 watts) and to a mechanical shaking method, indicates that significantly higher counts were consistently obtained after sonic vibration. It is likely that these differences are due to a more homogeneous dispersion of bacteria after sonic vibration.

2. The effects of sonic vibration on the counts may be noted as early as after 2 minutes of exposure, but the highest counts seem to be obtained after about 10 minutes. After reaching a maximum the counts become lower as the time of exposure is increased.

3. Similar effects may be noted on the numbers of colonies of streptococci and lactobacilli when aliquots from the sample are inoculated on separate media.

4. The possible use of sonic vibration, or some similar method for studying quantitative relationships between bacteria cultivable from saliva is suggested.

5. Various phases in the future study of the effects of sonic vibration on samples of saliva are discussed.

ADDENDUM

Dr. M. P. Horwood, Massachusetts Institute of Technology, states, in a personal communication, that he has observed results similar to ours when sewage or pure cultures of streptococci or staphylococci are exposed to ultrasound.

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DEVELOPMENTAL DISTURBANCES OF ENAMEL IN SCURVY

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THIS study deals with both scorbutic enamel changes and changes produced during the first stage of cure of scurvy in guinea pigs. In addition, some details of amelogenesis have been analyzed on the basis of the findings of these experiments. Some investigators[14, 31, 40] have reported that developmental disturbances of enamel in guinea pigs depend directly on scorbutic degeneration of the ameloblasts. Another group of authors[2, 7, 24, 39] have stated that the enamel formation is not primarily affected by ascorbic-acid deficiency.

Kotanyi[31] recognized two types of alteration in scorbutic enamel. In the first he found granular disintegration of the ameloblasts, enamel hypoplasia, and the development of hollow spaces between the enamel surface and the ameloblasts. To the other type belong cases where the ameloblasts are normal but the enamel shows separation into fibers and cysts. Walkhoff[40] established that deficiency of vitamin C directly effected the enamel-forming cells. The principal features in pathological degeneration of the ameloblasts consist of rapid reduction of their protoplasm and in development of vacuoles in the cells. This distortion of cells resulted in hypocalcification and, in severe cases, in hypoplasia of the enamel. Fish and Harris[14] emphasized that changes in the enamel-forming cells are characteristic of the scorbutic process. In complete scurvy the ameloblasts, which should be functioning at that moment, undergo permanent degeneration. Even those cells which have just developed degenerate and enamel formation ceases abruptly. If the animal is cured, there will never be any enamel on that section of the tooth which was forming when the disease was at its height.

Bauer,[2] as well as subsequent authors, belongs to the second group of investigators who consider that enamel development is not affected primarily by scurvy. He declared that in scurvy the origin of enamel hypoplasia was due to disturbances of circulation with edema of the enamel pulp. This edema results in injuries and in the separation of the ameloblasts from the enamel surface followed by cessation of enamel formation.

Boyle[9] stated that enamel hypoplasia was a secondary rather than a primary consequence of vitamin C deficiency. The development of defective enamel was explained with pathological changes in tooth suspension. In scurvy, the failure of collagenous fiber formation in periodontium which impedes the distribution of the masticatory stress to the base of the tooth allows the transmission of excessive forces to the formative part of the tooth, adequately accounting for the areas of defective enamel formation. Such areas, both in case of complete and partial vitamin C deficiencies, are commonly associated with hemorrhage in the overlying tissues and with disruption and atrophy of the enamel-forming cells.

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Kalnins[27] found that ascorbic acid deficiency does not influence the differentiation of the ameloblasts and that pathological changes of enamel are of a secondary nature. A characteristic feature of scorbutic changes of the enamel is insufficiency and interruption of enamel formation due to the cessation of the development of the dentin. This only applies to molars. Formation of the enamel of incisors, contrary to that in molars, continued without interruption in spite of full development of scurvy, because in these teeth the enamel tissue is adjacent to the "mantle dentin" which remains unchanged in vitamin C deficiency. The same observation was made by Wassermann[39] who stated that in certain areas of scorbutic teeth where the development of dentin was suppressed the enamel production also appeared to be interrupted. He emphasized that in such areas the enamel forming cells remain in an embryonic stage.

From this survey of the literature it will be seen that at present there is no possibility of drawing any uniform conclusions as to the pathogenesis of disturbance of enamel in experimental scurvy.* Therefore, one of the objects of the present work is to attempt to distinguish between primary and secondary scorbutic disturbance in the development of enamel.

MATERIAL AND METHODS

The investigation is based on studies of lower incisors and molars of about 250 guinea pigs with body weights of approximately 200 to 400 grams each. One hundred twenty animals are those of an earlier experiment[27] on the effect of ascorbic acid upon scorbutic teeth and jaws. The rest, about 130 guinea pigs, have been used as control animals in various experiments dealing with vitamin C[23, 24] with hormones,[29] periodontal disease,[25] and with the effect of x-ray radiation.[46] Two kinds of scorbutogenic diets were applied: one was Sherman Göthlin (A) and the other (B) consisted of:

Ground oats	500 parts
Wheat-bran	200 parts
Calcium lactate	10 parts
Sodium chloride	5 parts

As an antiscorbuticum l-ascorbic acid was chiefly used. Both in prophylactic and curative experiments 0.05 per cent, 0.1 per cent, or 0.5 per cent solutions were administered either per os, by stomach, or parenterally.

All experimental animals were divided into two groups. The changes of enamel in prescorbutus and in clinical scurvy were studied in 62 guinea pigs who had received only scorbutogenic food. The last 22 died in the course of the further development of severe scurvy. In the second group of 110 guinea pigs the enamel alterations during the period of reduction of the severity of scurvy, subscurvy, and the first stage of cure of scurvy were examined. These animals, according to the varied duration of scorbutic periods (13 to 23 days), were receiving, for 1 to 20 days, 0.1 to 3.0 and 25.0 mg. of ascorbic acid per animal per day.

*Moreover enamel changes in scurvy[15, 17, 19, 32, 34] due to a combination of scurvy and other disturbances[13, 16, 20, 42] have been briefly described by other investigators.

Moreover, the enamel was examined in 60 animals which in various curative experiments had been treated with doses of antiscorbuticum in the form of extracts or emulsions prepared from some animal tissues and organs[23, 24, 25] (liver, brain, kidneys, adrenals, tooth germs, etc.).

Since the ground sections of the jaws are not suitable for study of developing enamel, because the ameloblasts and enamel matrix are destroyed, decalcified sections were made. Only a few ground sections were made for the observation of sequences of developmental disturbances. The preparations were embedded in paraffin or, in some experiments, in celloidin, and then dissected in cross (incisors) and longitudinal planes (incisors and molars) 3 to 7 μ thick. Staining was with hematoxylin-eosin and according to Bock's or Hansen's methods.

Although, owing to the events of the war, the histologic preparations and many photomicrographs from the experiments performed before 1944 were lost, the photomicrographs of my work were completed by courtesy of Westin and Lilleengen from materials of their own experiments.* It should be noted that their methods with regard to animal diets and histologic preparations were similar to those of the present work.

RESULTS

The examination of molar teeth shows that after 18 to 22 days of basic diet, during the onset of manifest scurvy at the apical end of the tooth, the distortion of odontoblasts results in partial or complete cessation of dentin formation. Simultaneously, with this, the development of the corresponding layer of enamel matrix either diminishes or stops completely (Figs. 1, 2, 7, and 19). In these areas, the ameloblasts usually show no deviations of the polarization of their nuclei, nor in the demarcation between the ameloblasts and the cells of the stratum intermedium. Often the difference between scorbutic and normal ameloblasts lies only in the appearance in the cytoplasm of globular material in larger amounts than in the normal animals (Fig. 3). Apparently secretion of organic material continues, but, owing to the absence of dentin, its deposition is disturbed, and the product accumulates within the cytoplasm or at the basal ends of the cells (Fig. 2). This phenomenon may be called scorbutic dysfunction of ameloblasts. The thin layer of enamel matrix produced in the form of a loose chain represents the only barrier between the ameloblastic layer and the pulp (Fig. 1). Coronally, where enamel tissue is adjacent to dentin formed before the onset of scurvy, the growth and maturation of enamel continues in a normal way (Fig. 5) if it is not disturbed by other factors.

In guinea pigs, which are receiving small amounts of ascorbic acid (0.1 mg. per animal per day), the deposition of dentin is not completely interrupted and it develops in the form of collagen-free amorphous calcified layers of varied thickness. In these animals a hypoplastic formation of enamel matrix occurs instead of aplasia. In places where the irregular dentin is particularly thin, areas covered with enamel matrix layer usually show more

*See Figs. 5, 9, 13, 19, 21, and 23.

severe hypoplasia. The polarization of the cells and the demarcation between ameloblasts and stratum intermedium may not even show alterations (Fig. 4), just as it was in enamel aplasia of manifest scurvy.

In the course of the cure of scurvy a new portion of tooth develops. Here, in connection with the restoration of dentin formation, as early as the first or second day of treatment new enamel matrix develops. In the scorbutic tooth portion the ameloblasts which were not functioning resume, to some extent, the formation of enamel matrix (Fig. 9) simultaneously with the restoration of dentin production, although, at first, this may be in the form of osteocementum or osteodentin. The ameloblasts lying occlusally, however, remain in a state of dysfunction due to the fact that they have already attained either the stage in which they determine the maturation of the enamel or the period of senile involution. These phenomena are to be explained by the short and limited life span of the enamel-forming cells in the fast erupting teeth of rodents. If the therapeutic dose of vitamin C is small, or the sector of ameloblasts has been in dysfunction for a long time, due to a prolonged period of scurvy, there hardly occurs any resumption of formation of enamel matrix. Fractures of defectively formed tooth walls and the following complete destruction and disappearance of ameloblasts are common features of manifest scurvy in molars (Figs. 1 and 19). In the course of healing the ameloblasts distorted by these fractures do not recover and the corresponding areas of the tooth remain without enamel. These types of aplasia differ from those arising from dysfunction of ameloblasts in the appearance of coalescence between the dentin and the adjacent periodontal tissue or column of cartilage cementum (Figs. 10 and 13).

The findings in scorbutic incisors present a different picture. Here the formation of enamel matrix continues more or less without interruption. This is explained by the presence of "mantle dentin" in the incisors of the guinea pig.[27, 29] In molar teeth of normal animals, the predecessors of the dentinoblasts differentiate immediately into dentinoblasts which produce regular dentin. But in the incisors the antecedent cells, prior to their differentiation into dentinoblasts, form "mantle dentin." This kind of dentin is neither calcified nor canalized and represents the intermediate layer between the enamel and the regular dentin. Since in scurvy the unspecific mesenchymal cells of the dental papillae are less affected, in incisors the production of "mantle dentin," continues at approximately the normal rate. Thus, differently than in molars, the dentin wall, i.e., the base for the deposition of secreted organic material, has not completely vanished in scorbutic incisors and therefore the formation of the enamel matrix goes on more or less without interruption (Fig. 12).

In the course of the further development of manifest scurvy (after 20 to 24 days of basic diet), or the first stage of cure of scurvy, injuries of the ameloblasts take place in both molars and incisors, which depend first of all on the development of occlusal trauma and also on the deformation of the shape of the tooth wall.

The bleeding and the degeneration of the skeletal muscles are well-known features of scurvy.[11, 18, 20] The changes in masticatory muscles result in the reduction of the force of mastication and in subsequent retardation of the functional wear. As the harmony between the process of functional attrition on one side and the rate of the eruption and the length of the tooth on the other is essential for normal functioning of the continuously developing and erupting teeth of rodents, the disturbance of one of these factors must forcibly be followed by the disbalance of the two others.[30] Since, during manifest scurvy the rate of eruption is obviously less severely disturbed than the rate of the functional wear, the elongation of the crowns (Fig. 6) result in bite raising, i.e., occlusal trauma develops. In connection with this supra-occlusion the axis of tooth rotation relocates apically. But, although mastication is reduced, owing to the scorbutic distortion of muscles, the consequence of these pathological changes in the dissipation of the masticatory forces appears as an increase in the functional demand upon the alveolar bone. Since the scorbutic bone itself is also weakened, overstress results in the destruction of the alveolar bone on both the horizontal and vertical plane. In this way further elongation of the extra-alveolar portion takes place on account of the reduction of the height of the alveolar process. Moreover, owing to the severe destruction of the supporting apparatus, a buccal displacement[9] or the intrusion of teeth, sometimes occurs (Fig. 19).

Thus, on account of the occurrence of occlusal trauma, the apical relocation of the fulcrum of tooth movement, the widening of the periodontal membrane (due to the destruction of the alveolar bone in the vertical plane) as well as the failure of the collagen fiber formation of the periodontium[8] allows the transmission of excess masticatory forces into the formative part of the tooth, leading to damage in the developing enamel tissue.

A further increase of occlusal trauma occurs during the first stage of cure of scurvy which must be accounted for by the temporary maintenance of a raised bite. Apparently, the regeneration of the masticatory muscles damaged in scurvy takes much less time than the restoration of the normal height of the alveolar process (by means of the deposition of new bone) and of the height of the crowns (by means of the restoration of physiological abrasion). Therefore, the effect of occlusal trauma upon the tooth tissue is even more pronounced than in the final culmination of manifest scurvy.

The other factors which cause damage in the enamel tissue are the folding and bending of teeth, especially of molars. Bending of the tooth also takes place if it had been displaced in the period of scurvy and during healing its newly developing portion grows in the normal direction. Hence, the two portions of the tooth form an angle (Fig. 13). Finally a bending of the tooth also appears when the supply of vitamin C in small doses is sufficient for regeneration of the masticatory muscles, but insufficient for the complete development of dentin. Here, owing to the weakly developed dentin wall, the tooth bends under the forces of mastication (Fig. 15).

As consequences of this pathological dissipation of the masticatory forces and of the bending and folding of the tooth the following changes take place in the enamel: the detachment and disruption of the ameloblasts from the enamel matrix and partial or complete distortion of these cells in the form of a pressure atrophy with the subsequent development of hypoplastic or hypocalcified enamel.

Detachment of enamel-forming cells is a rare form of enamel tissue damage in manifest scurvy. As a rule, detachments take place in the area of growth of the enamel (fourth sector of Weinman). Since this detachment is produced chiefly by the bending of the tooth, it is observed more commonly in subscurvy or during the cure of scurvy (Fig. 11). If, in the course of the detachment, the ameloblasts are not damaged they continue to produce within the periodontium enamel matrix in the form of single or multiple larger or smaller drops of a globular or ellipsoid appearance. If, during the occlusal shifting (due to the fast eruption) the ameloblasts remain intact, these drops become mineralized at the same level where the enamel layer undergoes maturation (Fig. 8).* The areas of the enamel layer, where the detachment of ameloblasts with the subsequent formation of enamel drops takes place, show a more or less visible hypoplasia (Fig. 11). The longer the portion of the detached ameloblastic layer, the larger is the extent of hypoplasia. On the other hand, if the detachment is localized more apically, the hypoplasia is deeper.

Disturbances of amelogenesis, provoked by pressure atrophy of the ameloblasts, are of a much more severe character than the disturbances from the detachment mentioned above. According to the phase of development of the enamel tissue, these changes appear either as hypoplasia (Figs. 12 and 16), when the growing enamel is affected, or as enamel hypocalcification when the maturation of enamel is disturbed (Figs. 14 and 16). In some areas where the pressure apparently has not been so strong, and the ameloblasts have remained capable of functioning to a certain degree throughout the normal time of their formative activity, the maturation of hypoplastic enamel matrix takes place (Fig. 16). If the pressure is slight and affects only some groups of ameloblasts in the stage of enamel maturation, the mineralization of the enamel is delayed or only partly disturbed. In such cases the portion of enamel matrix adjacent to dentin remains uncalcified, whereas the part covered with ameloblasts undergoes maturation (Fig. 17). If, occasionally, during the healing the tooth bending, brought about by the excess of functional demand, or by pressure, was particularly grave, osteoclastic resorption of the already matured enamel takes place (Fig. 18). Since, in the incisors, the ratio between the length of the crown and the length of the embedded portion is much more favorable than in molars and, moreover, since the lower incisor normally is bent in two planes[42] its enamel tissue is not so severely affected by occlusal trauma as that of the molars. Therefore, hypoplasia and hypocalcification of enamel caused by occlusal trauma and by distortion of the curvature, are less pronounced in incisors than in molars (Fig. 12).

*The details of formation of enamel drops will be described in another work.[38]

Finally, if the tooth is depressed in the alveolus the balance between the rate of enamel formation and the eruption of the tooth becomes disproportionate. In this case, both maturing enamel and senescent ameloblasts are prevented from moving up in the regular way. The consequence of all this is that mature enamel and short ameloblasts appear in more apical portions than normally, which makes the impression of "premature amelogenesis" and advanced degeneration of the enamel forming cells (Fig. 19).

DISCUSSION

The most common scorbutic enamel changes may be classified as follows:

1. Aplasia and hypoplasia, which appear in consequence of the dysfunction of the ameloblasts, caused by the reduction and the complete cessation of dentin development. These changes are observed only in molars.

ABBREVIATIONS FOR FIGURES

Am, ameloblasts; *E*, enamel matrix or enamel (i.e., space formerly occupied by enamel now lost in decalcification); *d*, dentin; *ab*, alveolar bone; *cc*, cartilage cementum. All photomicrographs were taken from preparations stained with hematoxylin and eosin (with exception of Figs. 6 and 10) and, with the exception of Figs. 6, 10, and 12, present a formative part of molar teeth in longitudinal sections.

Fig. 1.—Aplasia and hypoplastic formation of enamel matrix during manifest scurvy due to reduction and cessation of dentin development (22 days B-diet). In lower half, thin layer of enamel matrix is produced only in form of loose chain. Note normal polarization of nuclei of ameloblasts in this portion and disappearance of distorted ameloblasts at area (-x) of fracture of tooth wall. Orig. mag., 200×.

Fig. 2.—Ameloblasts in scorbutic dysfunction. Note the accumulation of organic material at basal end of cells and normal polarization of nuclei. Enlarged microphoto from ameloblasts of lower part of Fig. 1. Orig. mag. 600×.

Fig. 3.—Normal differentiation of ameloblasts in area of aplasia of enamel matrix during manifest scurvy (20 days A-diet). Globular material in cytoplasm of ameloblasts appears in greater amounts than normally. Note normal polarization of nuclei. Orig. mag. 900×.

Fig. 4.—Hypoplastic formation of enamel matrix due to defective development of dentin during reduction of manifest scurvy (20 days A-food) by 0.1 mg. ascorbic acid daily (20 days). Note normal demarcation between ameloblasts and stratum intermedium. Orig. mag. 160×.

Fig. 5.—Normal maturation of enamel matrix in portion adjacent to dentin with amorphously calcified predentin. Manifest scurvy (A-diet). Orig. mag. 90×.

Fig. 6.—Maxilla of two guinea pigs of same age. Pronounced elongation of crowns (x) of molars (Fig. 6b) during manifest scurvy (A-diet 25 days) in comparison with usual height of crowns of molars of normal guinea pig (6a). Alveolar process is stained black. Orig. mag. 3×.

Fig. 7.—Aplasia of enamel matrix occurred owing to temporary cessation of dentin formation at area-x. Manifest scurvy. Orig. mag. 200×.

Fig. 8.—Calcified free enamel drop. Note presence of ameloblasts adjacent to drop. Orig. mag. 200×.

Fig. 9.—Resumption of formation of enamel matrix during cure of scurvy (22 days A-food + 4 days A-food with daily additions of 5 cm. of fresh orange juice). a-scorbutic sector of molar tooth with richly developed "healing" osteo cement (d) and newly but insufficiently formed enamel matrix. b-developed sector of tooth in treatment. Orig mag. 160×.

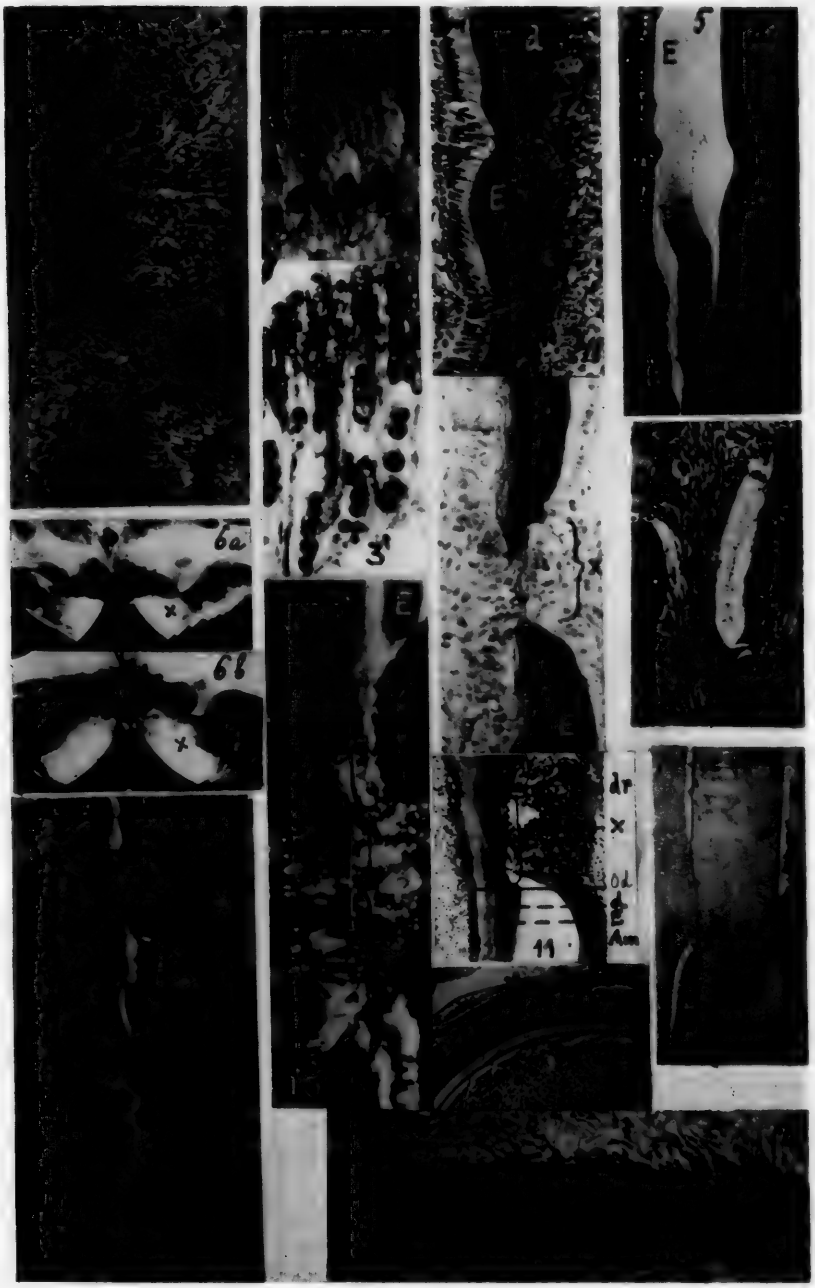
Fig. 10.—Ground section of scorbutic molar, from section with calcified enamel after a long period of cure of scurvy (20 days A-food + 20 day A-food with addition of 2.5 mg. ascorbic acid daily). In sector (a) enamel aplasia took place owing to dysfunction of ameloblasts, but in sector (b) due to fracture of tooth wall in scorbutic period. Orig. mag. 60×.

Fig. 11.—Detachment and disruption of ameloblasts from growing enamel matrix. Detached ameloblasts continue to produce enamel matrix in form of free enamel drops (-dr) in periodontium. Hypoplastic formation of layer of enamel matrix in area of detachment (x). Subscurvy (18 days A-food + 12 days A-food with the addition of 4 c.c. brain emulsion, doses which correspond to about 0.4 mg. ascorbic acid). Orig. mag. 70×.

Fig. 12.—Continued formation of enamel matrix in spite of manifest scurvy (20 days B-food) due to presence only of normally developed "mantle dentin" in incisor. Hypoplasia (-x) is caused by the transmission of masticatory forces in formative part of tooth (cross section).

Fig. 13.—Appearance of angle between scorbutic (a) and newly developed (b) (during the cure of scurvy) portions of molar teeth. Note aplasia of enamel at apical end of scorbutic portion, and at points-x coalescense between dentin and periodontium owing to fracture of tooth wall in scorbutic period. Orig. mag. 20×.

Fig. 14.—Pressure atrophy of ameloblasts and absence of maturation of enamel matrix due to transmission of masticatory forces into formative zone of molar tooth, in manifest scurvy. Orig. mag. 200×.



(For legends see opposite page.)

2. Hypoplastic formation of enamel matrix in molars or incisors, due to the detachment, pressure atrophy or distortion of the ameloblasts during growth. Here the etiological factor is either occlusal trauma or the bending and folding of the tooth wall.

3. Partial or complete disturbances of maturation of enamel in molars or incisors, caused by occlusal trauma, of the bending of the tooth, affecting the ameloblasts during maturation.

The retardation and the subsequent complete cessation of dentin formation is a typical phenomenon of severe scurvy and up to now it has not been observed in other vitamin or hormonal disturbances. In connection with this, enamel hypoplasia and aplasia, caused by the reduction and the cessation of dentin growth, which appears at first in scurvy and only in molars, must be considered as the primary and typical feature of the scorbutic disturbances of enamel. Furthermore, Figs. 1, 2, 3, and 4 show that, notwithstanding, the scorbutic dysfunction resulting in inability to form enamel matrix, the ameloblasts generally develop in the usual way as far as their secretory potentiality and polarization of nuclei are concerned. If, in accordance with Wassermann's statement, the embryonal development of ameloblasts is stopped by scurvy (due to the cessation of dentin formation), it ought to be presumed that during the cure of scurvy the ameloblasts would resume their complete function of forming enamel matrix within the whole area of scorbutic aplasia. Fig. 9 shows that this was not the case.

The observations described demonstrate that the differentiation and the secretory activity of the ameloblasts in scurvy are not disturbed but that it is only the possibility of producing enamel matrix that is lost owing to the absence of dentin, i.e., of the base for the deposition of organic material.

Fig. 15.—Bending of molar wall under forces of mastication due to insufficient formation of dentin in subscurvy (10 days A-food + 10 days A-food with the addition of 0.25 mg. ascorbic acid daily). Disturbances of enamel and osteoclastic resorption of alveolar bone caused by pressure. Orig. mag. 40×.

Fig. 16.—Hypocalcification and hypoplastic formation of enamel matrix, due to pressure atrophy of ameloblasts caused by transmission of masticatory forces in formative part of tooth. Upper third shows sector where hypoplastic enamel matrix became matured owing to persistence of ameloblasts. Remaining part of hypoplastic enamel matrix remained uncalcified owing to complete distortion of ameloblasts. Subscurvy (10 days B-food + 14 days B-food with the addition of 0.25 mg. ascorbic acid daily). Orig. mag. 200×.

Fig. 17.—Delay of maturation of enamel matrix due to transmission of masticatory forces into formative portion of molar tooth. Complete absence of mineralization in areas (x) of attachment of periodontal fibres. Between them only outer enamel layer covered with ameloblasts is mineralized. Manifest scurvy. Orig. mag. 400×.

Fig. 18.—Osteoclastic resorption of mature enamel, caused by pressure, due to bending of molar tooth during cure of scurvy (20 days A-food + 14 days A-food with the addition of 1 mg. ascorbic acid daily). Orig. mag. 400×.

Fig. 19.—Appearance of "premature amelogenesis" due to intrusion caused by mastication forces owing to defectively formed tooth wall of molar. Manifest scurvy after 23 days of B-diet. Note numerous fractures (x) of folded tooth wall and hypoplastic formation of enamel matrix due to cessation of dentin development. Orig. mag. 40×.

Fig. 20.—Artifact: "Separation of enamel in fibres." Growing enamel matrix of molar of normal guinea pig. Orig. mag. 120×.

Fig. 21.—Artifact: "Edema of the enamel organ" (x) in molar of normal guinea pig.

Fig. 22.—Artifact: "Appearance of globular material in dentin" in molar of normal guinea pig. Orig. mag. 600×.

Fig. 23.—Artifact: "Granular disintegration of ameloblasts" in the molar of normal guinea pig. Orig. mag. 400×.

(For legends see opposite page.)

Since disturbances of enamel growth and maturation caused by the development of occlusal trauma, accompanied by dissipation of masticatory forces in the formative portion of the tooth and by bending and folding of the tooth wall, have also been observed in experiments with hormone,[12, 37, 38] with mechanical damage[1, 30] of the tooth tissue, and with other vitamins,[3, 5, 10, 21, 22, 35, 45] than ascorbic acid, they cannot be regarded as typical for scurvy. The features of disharmony between the process of eruption and that of enamel formation are not characteristic either because they have also been observed in experiments with capping[30] and hypophysectomy.[4, 36] As, moreover, these atypical enamel disturbances appear in a state of scurvy later than the dysfunction of ameloblasts, they may be considered as secondary scorbutic enamel changes.

Scorbutic alterations described by other research workers,[2, 31, 39, 40] such as separation of enamel into fibers, granular disintegration, and vacuolar degeneration of ameloblasts, edema of the enamel organ and appearance of globular material in dentin, seem to be artifacts which are brought about in a way similar to the artifacts in dentin and pulp tissues produced by various forms and mechanisms of shrinking in the course of histological preparations.[28] These artifacts of enamel can also be demonstrated in teeth of normal animals (Figs. 20 to 23).

Some of the previous investigators of scorbutic changes in teeth of guinea pigs came to the conclusion that vitamin C deficiency in man would cause the failure of enamel formation and hereby might be significant in the etiology of caries. The results of the present work show that in scorbutic guinea pigs aplasia and hypoplasia of enamel take place only when the development of dentin is almost, or completely, stopped. Since, up to the present day such severe forms of scorbutic dentin disturbances have not been observed in man it seems doubtful that scurvy should be the etiological factor in the origin of human enamel hypoplasia. In this connection, it is of interest to remember that Boyle,[6] during his investigation of the tooth germ in infantile scurvy and upon examination of permanent teeth in a limited number of patients who had recovered from infantile scurvy, found that enamel hypoplasia did not result from ascorbic-acid deficiency during the period of tooth formation. Also, Westin,[44] in his study of scurvy in human patients, did not find any case of scorbutic enamel changes.

Our findings allow us to arrive at some conclusions on the function of the ameloblasts in the stage of enamel maturation. The mineralization of enamel drops freely located in the periodontium (Fig. 8) as well as normal enamel maturation in the areas of scorbutic dentin, which, owing to the amorphous calcification of predentin, is sealed off from the pulp (Fig. 5) show that contrary to the assumption of other investigators[39] no additional uptake of minerals via the dentinoenamel junction takes place during the maturation period. Moreover, the delay of enamel maturation in the scorbutic tooth, where only the ameloblast-covered part of the enamel matrix is mineralized, shows that the histological pictures cannot serve as evidence for the correct-

ness of the statement of other investigators that the enamel maturation first becomes visible at the dentinoenamel junction (Fig. 17). These factors allow us to conclude that the indispensable function of the ameloblasts during the process of enamel maturation must consist, not only in the removal of the fluid of organic substances, but also in the additional uptake of minerals into the enamel matrix.

SUMMARY

Developmental disturbances of enamel in scurvy and in the first stage of cure of scurvy were studied in molars and incisors of a large number of guinea pigs.

1. The first and characteristic change of enamel in scurvy is dysfunction, i.e., the inability of the ameloblasts to produce enamel matrix (aplasia and hypoplasia) due to the retardation and complete cessation of the development of dentin in the molars.

2. The secondary and nontypical scorbutic changes are the disturbances of formation (hypoplasia) and of maturation (hypocalcification) due to the detachment or pressure atrophy of the ameloblasts caused by the bending of the tooth or by the transmission of the masticatory forces into the formative portion of the tooth. These changes can be observed both in molars and in incisors.

3. The additional uptake of minerals into the enamel during the maturation phase is determined by the activity of the ameloblasts, not by the function of the pulp through the dentinoenamel junction.

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